

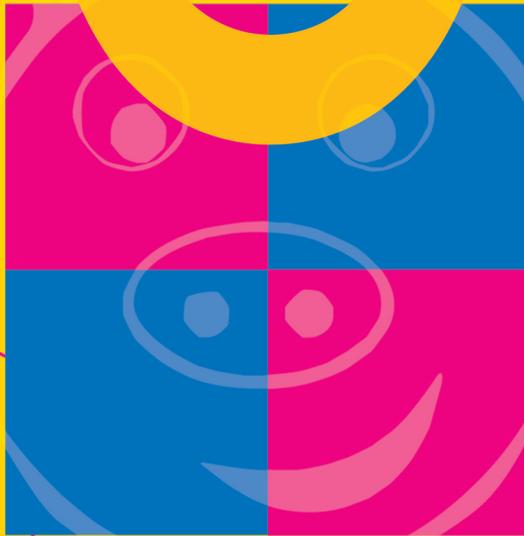
7th

SAFE PORK

INTERNATIONAL SYMPOSIUM

ON THE EPIDEMIOLOGY AND CONTROL
OF FOODBORNE
PATHOGENS IN PORK

OCTOBER 1-4, 2003



CRETA MARIS HOTEL

HERONISSOS, HERAKLION - CRETE • GREECE

Proceedings

SAFE PORK 
.....

**5th International Symposium on the Epidemiology
and Control of Foodborn Pathogens in Pork**

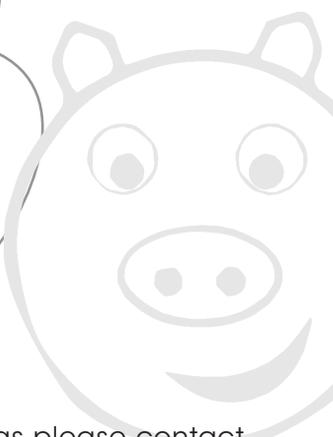
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PROCEEDINGS
.....

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School of Veterinary Medicine, University of Thessaly
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For information on purchase of the Symposium proceedings please contact
the e-mail address: leoleont@vet.uth.gr





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WELCOME ADDRESS

It is a great pleasure to welcome all of you at the 5th International Symposium on the Epidemiology and Control of Foodborne Pathogens in Pork, in Greece.

The program of this symposium brings forward many important issues concerning public health. The supply of quality assured pork is the prerequisite for consumer confidence. As it was clearly shown from the recent crises of the beef industry, the loss of the consumer's confidence may result to a shift in its choices, resulting to serious losses for the beef meet sector.

The list of the Symposium participants, from all over the world, shows that many countries share these views and that the international scientific community is actively seeking for practicable ways to resolve the very complex interactions between pathogens and farmed pigs. The production of "sterile" pork may not be technically feasible but there is no reason for not taking every possible measure to lower the risks to consumer's health to the lowest level possible.

We are looking forward to the fruitful exchange of scientific opinions and likely recommendations from your meeting as consumers and decision makers expect from the public health scientists to provide innovative and readily applicable strategies that ensure the safety of pork through the whole chain of production.

Fotis Xatzimichalis
Deputy Minister of Agriculture of Hellas

WELCOME ADDRESS

Foodborne diseases remain a major human health problem that is on the rise world-wide despite of extensive knowledge and efforts to improve food hygiene and food control services in many countries. Their economic impact is enormous and greatly affects both society and individuals. Countries like ours with tourism being a key contributor to economic growth are especially vulnerable to crisis and therefore we are addressing food safety issues very seriously.

The stable to table concept as an integrated food safety program intends to minimize the threat of a crisis by addressing specific problems and solutions at the individual links and determining their interactions and impact on other links as well as on the entire food chain. Therefore national food safety policies should aim on improvements and solutions at both the preharvest and post harvest level of production and consumer education. No single Agency or group of people can address effectively the problems of all links in the chain unless it is multidisciplinary in nature and keeps communication channels open at all times and to all concerned. At the preharvest level in many countries the concepts of traditional veterinary preventive medicine need modernization toward the direction of population medicine, HACCP, ISO and disease surveillance focusing also on foodborne pathogens. As a medical doctor I also realize that at the consumer level we need effective surveillance and alert systems to discover, early enough, foodborne disease outbreaks and especially to make sense out of sporadic cases. Remember that for every recorded human case there are 25-350 additional escaping our detection system today. Early warning signals forwarded through an integrated food safety and traceability system to the rest of the food chain links be them regulatory agencies, producers, industry, media and consumers will result into a realistic risk assessment, prevention or elimination of a food safety crisis.

The title of this Symposium <<Safe Pork>> might be misleading to some as far as its focus. Yet the number and diversity of the papers submitted, in terms of new knowledge and concepts, go far beyond the commodity of pork. They will definitely have a serious impact on the safety of all foods of animal origin which are the most frequently incriminated sources of foodborne disease.

My colleagues and I are wishing all of you a successful and rewarding Symposium and a pleasant stay in Crete.

Dr Christina Papanikolaou, MD Biopathologist
Chair of the Board Hellenic Food Authority – EFET
National Representative in the Advisory Scientific
Forum of European Food Safety Authority – EFSA

WELCOME ADDRESS

Dear Colleagues,

It is my privilege to welcome you to Greece!

Food safety is one of the most important challenges confronting human health around the world. Consumers are demanding close monitoring and control of health hazards that are related to the entire chain of food production. In addition, they want public health scientists to provide innovative risk-based control strategies that can be readily applied to animal populations on the farm and to the rest of the food chain as well. In the past, the industry was largely concerned with the expansion of pig-production. An increase in productivity is, however, challenged today by the consumers' skepticism about the quality of the pork produced. The previous Symposia in Ames, Iowa (1996), Copenhagen (1997) and Washington DC (1999) were devoted to the methodology and strategies for control of Salmonella on pork. In Leipzig (2001) the scientific objectives were expanded to encompass other microbial and parasitic foodborne hazards that are transmitted via pork and its products. The Scientific Committee of the 5th Symposium decided to change the acronym of the Symposium from SALINPORK to SAFEPORK in order to encourage all public health scientists working in the field of quality assurance of pork and its products from the farm to the table to contribute to the Symposium, either by submitting a paper or poster for presentation or by participating actively in the discussions of the diverse topics that are going to be covered.

The scientific program has been structured to cover ten topics: 1) Microbial and parasitic ecology (pre- and post-harvest), 2) Pathogenesis, 3) Epidemiology, 4) Resistance, 5) Diagnostics, 6) Free-range and organic farming, 7) Slaughtering and processing of pork, 8) Intervention measures, 9) Human health implications, 10) Animal handling. There will be six invited presentations covering selected subjects, 86 oral and 50 poster presentations. The contributors represent 26 countries from all around the globe. In order to enable more vivid discussions, the Organizing Committee has scheduled the presentations in parallel sessions. In structuring the program the President of the Organizing Committee put special attention to prevent concurrent presentations in parallel sessions of papers that were grouped by the Scientific Committee under the same topics, thereby increasing the opportunities for participants to attend several papers in their area of scientific expertise.

With the exception of the keynote papers all other papers included in this book of proceedings were evaluated as preliminary abstracts by at least 2 members of the Scientific Committee and myself. Due to time constraints, I reviewed and edited all the final papers. Therefore, I am the only one responsible for any mistakes or omissions.

I hope that you will all enjoy the social program and the Greek philoxenia (hospitality)!

Leonidas Leontides
Congress President

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ACKNOWLEDGEMENTS

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SCIENTIFIC PROGRAM



SCIENTIFIC PROGRAM

WEDNESDAY, OCTOBER 1, 2003

08:30-21:00 Registration

THE THIRD ANNUAL GENERAL MEETING OF THE EUROPEAN COLLEGE OF VETERINARY PUBLIC HEALTH

HALL SECRETARIAT

10:00-12:50 8th Meeting of Interim Board of ECVPH

14:00-16:00 Annual General Meeting HALL ZEUS A & B

WORKSHOP: MATHEMATICAL MODELLING ON THE SAFETY AND SPOILAGE OF MEAT

(In the context of EU funded project: Development and Application of a TTI based Safety Monitoring and Assurance System for Chilled Meat Products- SMAS)
HALL ZEUS A & B

09:00-09:05 Welcome:

P. Taoukis and C.Genigeorgis (Greece)

09:05-09:45 Microbial modelling fundamentals

J. Baranyi (UK)

09:45-10:10 Mathematical modelling of safety and spoilage of meat

K. Koutsoumanis (Greece)

10:10-12:50 Presentation and demonstration of Combase,
a comprehensive microbial modelling tool

J. Baranyi and co-workers (UK)

12:50-13:15 Presentation of SMAS project

P. Taoukis (Project coordinator, Greece)

OPENING CEREMONY

HALL ZEUS A & B

19:00-19:10 Opening ceremony

L. Leontidis (Congress President)

19:10-20:00 Opening Speeches

20:00-20:30 Opening Lecture: Industry perspective on food safety:
More questions than answers

C. Schultz-Kaster, Bradley M. (USA)

20:30-21:00 Opening Lecture:

P. Willeberg (Denmark)

21:00-22:00 Welcome Reception

THURSDAY, OCTOBER 2, 2003

HALL ZEUS A

Epidemiology

09:00-11:00

**Keynote lecture: Evaluation of diagnostic methods
used in zoonosis-surveillance programs**

Enøe C., Boes J., Nielsen B. (Denmark)

SCIENTIFIC PROGRAM

THURSDAY, OCTOBER 2, 2003

- O01 **Sensitivity of cultivation of *Salmonella Enterica* in pooled samples of pig faeces**
Enoe C., Boes J., Dahl J., Svensmark B. (Denmark)
- O02 **Surveillance of zoonotic bacteria in finishing pigs in The Netherlands**
Van de Giessen A.W., Bouwknecht M., Dam-Deisz W.D.C., Wannet W.J.B., Nieuwenhuis M., Graat E.A.M., Visser G. (The Netherlands)
- O03 ***Campylobacter* prevalence in Danish finishing pigs from mixed production herds**
Boes J., Nersting L., Kranker S., Enoe C., Wachmann H. (Denmark)
- O04 **Eliminating the abattoir pen lairages to decrease the prevalence of salmonella in cull sows**
Larsen S.T., McKean J.D., Hurd H.S., Wesley I.V. (USA)
- O05 **Appearance of multiresistant *Salmonella* Typhimurium DT104 in swine in Poland**
Wasyl D., (Poland) Baggesen D.L., Sandvang D., Skov M.N. (Denmark)
- O06 **Molecular epidemiology of *Salmonella enterica* and subtyping using phenotypic and genotypic approaches**
Gebreyes W.A., Thakur S., Funk J.A. (USA)
- O07 ***Campylobacter* prevalence and diversity in antimicrobial free and conventionally reared market swine**
Gebreyes W.A., Bahnsen P.B., Funk J.A., Morgan Morrow W.E.M., Thakur S. (USA)

11:00-11:30 Coffee Break

HALL ZEUS A

Epidemiology

11:30-13:30

- O08 **Trends in salmonella shedding by U.S market hogs, swine 2000**
Bush E., Fedorka-Cray P.J (USA)
- O09 **Risk factors for *Yersinia enterocolitica* on U.S. swine farms in 2000**
Bush E., Wesley I., Bhaduri S. (USA)
- O10 **The sero-prevalence of *salmonella* spp. in finishing swine in Iowa**
O'Connor A.M., McKean J.D., Beary J., Brockus S., Zhou E. (USA)
- O11 **Risk factors for swine infection with *toxoplasma GONDII***
Pyburn D.G., Patton S., Zimmerman J.J., McKean J.D., Evans R.B., O'Connor A.M., Smedley K.L., Faulkner C.T., Zhou E.M. (USA)

SCIENTIFIC PROGRAM

THURSDAY, OCTOBER 2, 2003

- O12 Individual effect of the steps preceding slaughtering on *salmonella* contamination of pigs
Fravalo Ph., Cariolet R., Queguiner M., Salvat G. (France)
- O13 Epidemiology of salmonellosis in fattening units of Catalonia (Spain): a bacteriological survey
Mejia W., Zapata D., Martin M., Casal J., Mateu E. (Spain)
- O14 Epidemiology of salmonellosis in sow units of Catalonia (Spain)
Mejia W., Zapata D., Martin M., Casal J., Mateu E. (Spain)
- O15 The prevalence and risk factors of porcine cysticercosis in Zambia
Phiri I.K., Sikasunge C.S., Siziya S., Gabriel S. (Zambia),
Dorny P. (Belgium), Willingham A.L. III (Denmark)

13:30-15:00 Lunch break

HALL ZEUS A

Epidemiology

15:00-17:00

- O16 Serological research of *Salmonella* on Belgian pig Farms
Huysmans K.A., Nollet N., Vandebroeck M., Desmedt K., Geers R. (Belgium)
- O17 Risk factors for the prevalence of *Salmonella* in Belgian slaughter pigs
Nollet N., Maes D., Duchateau L., Huysmans K., Geers R.,
de Kruif A., de Zutter L., Van Hoof J. (Belgium)
- O18 *Yersinia* prevalence in antibiotic free and conventionally reared swine
Funk J.A., Bahnson P.B., Gebreyes W.A., Morgan Morrow W.E. (USA)
- O19 Tuberculous lesions in pigs in the Czech Republic in the years 1990-1999
Pavlik I., Matlova L., Dvorska L., Bartl J., Oktabcova L.,
Docekal J., Parmova I. (Czech Republic)
- O20 Genetic relatedness of *Salmonella enterica* isolates from pens and swine at slaughter
O'Connor A.M., Gray J.T., Hurd H.S., McKean J.D., Rostagno M.H. (USA)
- O21 *Salmonella* surveillance trends in porcine salmonellae in GB: 1996-2002
Cassar C., Speed K., Bennett G., Davies RH. (UK)
- O22 Investigations of potential transfer of *Campylobacter Coli* between hogs and turkeys
Lee B.C., Carver D.K., Kathariou S.

SCIENTIFIC PROGRAM

THURSDAY, OCTOBER 2, 2003

- 17:00-17:30 Coffee Break
- 17:30-19:00 Visiting Posters for Topics Epidemiology (PE),
Diagnostics (PD), Human health implication (PH)
- 20:00 Departure for Cretan Night

HALL ZEUS B

Diagnostics

09:00-11:00

- O23 Comparison of two commercial ELISA kits and bacteriology for *salmonella* monitoring in pig herds
Davies R.H., Heath P.J., Coxon S.M., Sayers A.R. (U.K)
- O24 Discrimination of vaccinated and infected pigs by *Salmonella*-Specific Iga Antibodies
Lehmann J., Rosler U., Lindner T., Kramer T., Gabert J., Hensel A. (Germany)
- O25 Expression study by real-time quantitative RT-PCR of the *Salmonella* Tympimurium mntH gene
Botteldoorn N., Werbrouck H., Rijpens N., Van Coillie E., Heyndrickx M., Herman L. (Belgium)
- O26 Development of a new molecular typing method of salmonella spp. based on SNPS detection
Van Bost S., Ghafir Y., Daube G., China B. (Belgium)
- O27 Correlation between bacteriology of lymph nodes and serology for *Salmonella* diagnosis in slaughter pigs
Nollet N., Huysmans K., Maes D., Houf K., Imberechts H., de Kruif A., de Zutter L., Van Hoof J., Geers R. (Belgium)
- O28 Effect of different enrichment media and DNA extraction techniques on *salmonella* detection by PCR in swine feces
Oliveira C.J.B., Freschi C.R., Carvalho L.F.O.S. (Brazil)
- O29 Development of an *In Vitro* method for detection of *clostridium botulinum* types A and E using real-time PCR
Artin I., Lövenklev M., Rådström P., Holst E. (Sweden)
- O30 Sampling cecal contents or ileocecal lymph nodes: Is it different?
Rostagno M.H., Hurd H.S., Gailey J.K., McKean J.D. (USA)

11:00-11:30 Coffee Break

SCIENTIFIC PROGRAM

THURSDAY, OCTOBER 2, 2003

HALL ZEUS B

Diagnostics

11:30-12:15

- O31 Proficiency test of four *Salmonella* antibody ELISA - tests for their harmonization
Blaha Th., Ehlers G., Methner U., Leyk W., Rohn K., Kreienbrock L. (Germany)
- O32 Abbreviated identification scheme for *escherichia coli* in swine feces
Mack A., Funk J. (USA)
- O33 Isolation of *Salmonella enterica* in seropositive classified finishing pig herds
Lo Fo Wong D.M.A., Dahl J., Van der Wolf P.J., Wingstrand A. (Denmark), Leontides L. (Greece), Von Altrock A. (Denmark)

Human Health Implications

12:15-13:30

- O34 Pork and the number of human multi-resistant *Salmonella* Typhimurium DT104 cases
Alban L., Dahl J. (Denmark)
- O35 Estimated society costs for pork-related *Salmonella* and *Yersinia* in Denmark in 2002
Nielsen B., Korsgaard H.B. (Denmark)
- O36 Comparison of *Campylobacter* Coli strains isolated from pigs and humans-porcine strains a possible source of human infection?
Gurtler M., Alter T., Kasimir S., Fehlhaber K. (Germany)
- O37 Pathogenic bacteria and indicator organisms for antimicrobial resistance in pork meat at retail level in The Netherlands
Van der Zee H., Wit B., De Boer E. (The Netherlands)
- O38 Antibiotic susceptibility and setotyping as epidemiological tool for endemic salmonella spp.in raw and processed pork
Kuri V., Collins M.A., Madden R.H. (UK)
- O39 Preharvest Influence on Salmonellae Human Health Costs and Risks from Pork
Miller G., McNamara P., Liu X. (U.S.A)

13:30-15:00

Lunch break

THURSDAY, OCTOBER 2, 2003

HALL ZEUS B

Pathogenesis

15:00-15:45

- O40** Performance of Anti-*Salmonella* lactic acid bacteria in the porcine intestine
Gardiner G.E., Casey P.G., Casey G., Lynch P.B., Lawlor P.G., Hill C., Fitzgerald G.F., Stanton C., Ross R.P. (Ireland)
- O41** Survival of *Salmonella* serovar Typhimurium inside porcine monocytes is associated with complement binding and suppression of the production of reactive oxygen species
Donne E., Pasmans F., Ducatelle R., Haesebrouck F. (Belgium)
- O42** The stomach acts as a barrier against *Salmonella* in pigs fed a meal diet
Hansen C.F., Mikkelsen L.L., Bach Knudsen K.E., Jensen B.B. (Denmark)

Free-range and Organic farming

15:45-16:00

- O43** Zoonotic pathogens and antimicrobial resistance in "animal-friendly" pig production systems in Switzerland
Ledergerber U., Regula G., Danuser J., Bissig B., Stephan R., Stark K.D.C. (Switzerland)
- O44** *Campylobacter* species distribution in outdoor pigs
Jensen A.N., Nielsen E.M. (Denmark)

17:00-17:30 Coffee Break

17:30-18:30 Visiting Posters for Topics Epidemiology (PE), Diagnostics (PD), Human Health Implications (PH)

20:00 Departure for Cretan Night

SCIENTIFIC PROGRAM

FRIDAY, OCTOBER 3, 2003

HALL ZEUS A

Intervention Measures

09:00-11:00

Keynote lecture: Ensuring the safety of animal feed

Notermans S., Beumer H. (Netherlands)

Keynote lecture: Fermented Liquid Feed (FLF) can reduce the transfer and incidence of Salmonella in pigs

Brooks P.H., Beal J.D., Demeckova V., Niven S.J., (U.K.)

- O45 **Effect of an optimised pelleted diet on *Salmonella* prevalence and pig productivity**
Jorgensen L., Boes J., Kranker S., Kjaersgaard H., Wachmann H. (Denmark)
- O46 **Effect of feeding strategy on Salmonella in Danish sows and weaners**
Bysted D. (Denmark)
- O47 **Feeding fermented liquid feed to the gestating sow can reduce pathogen challenge of the neonatal environment**
Demeckova V., Tsourgiannis C.A., Brooks P.H. (U.K.)
- O48 **A randomised controlled trial to reduce *Salmonella* infection in finisher pigs**
Cook A.J.C., Davies RH, Miller AM, Gopal R, Byrne C, Heath P.J., Cousens S (U.K.)

11:00-11:30 Coffee Break

Intervention Measures

11:30-13:30

- O49 **Selection of finishing pig herds with a low Salmonella prevalence for logistic slaughtering**
Van der Wolf P.J., Swanenburg M., Urlings H.A.P., Snijders J.M.A. (Netherlands)
- O50 **Cost-effectiveness of *Salmonella* control in the pork chain using maximum acceptable prevalence levels**
Van der Gaag M., Saatkamp H., Van Beek P., Huirne R. (The Netherlands)
- O51 **Control of Salmonella at pig finishing farms with a farm decision tree**
Mul M., Van der Gaag M., (The Netherlands)

SCIENTIFIC PROGRAM

FRIDAY, OCTOBER 3, 2003

- O52 **Mixed culture of commensal bacteria reduces *E. Coli* in nursery pigs**
Harvey R., Genovese K.J., Anderson R.C., Nisbet D.J. (USA)
- O53 **Trichinae certification in the United States pork Industry**
Pyburn D.G., Gamble H.R., Anderson L.A., Miller L.E. (USA)
- O54 **Fermented Liquid Feed: The potential for eliminating enteropathogens from feed**
Beal J., Moran C., Brooks P. (U.K.)
- O55 **Population of a farrowing unit by *Salmonella* negative animals**
Letellier A., Bonneau M., Michaud C., Messier S., Quessy S. (Canada)
- O56 **Field trials to evaluate the efficacy of mash feed to reduce *Salmonella* shedding in swine**
Letellier A., Ménard J., Quessy S. (Canada)
- 13:30-15:00** Lunch break at the Conference Venue
- 15:00-17:00** Visiting Posters for Topics Intervention Measures (PI), Resistance (PR), Slaughtering and Processing of Pork (PS), Pathogenesis (PP)
- 17:00-17:30** Coffee Break
- 17:30-19:00** Poster Discussions for Topics Epidemiology (PE), Human Health Implications (PH)
- 21:00** Farewell Dinner by the beach

HALL ZEUS B

Slaughtering/processing/handling

09:00-11:00

- O57 **Pilot experiment with the aim to reduce *Salmonella* prevalence in pork by logistic slaughter of pigs**
Swanenburg M., Van der Wolf P.J., Urlings H.A.P., Snijders J.M.A. (The Netherlands)
- O58 **Does animal origin and hide status affect microbial contamination in pig carcasses?**
Travisani M., Marzadori F., Rosmini R., Rustischelli R. (Italy)
- O59 **The intensified control programme for *Salmonella* at Danish swine slaughterhouses**
Sorensen L.L. (Denmark)
- O60 **Exposure assessment of foodborne pathogens in pork in Belgium**
Ghafir Y., De Zutter L., Francois J.Y., Cornelis M., Jouret M., Dumont J.M., Dierick K., Daube G. (Belgium)

SCIENTIFIC PROGRAM

FRIDAY, OCTOBER 3, 2003

- O61 Improving the meat inspection by an integrated quality control system
Snijders J. (The Netherlands)
- O62 Prevalence of *Listeria monocytogenes* and *Listeria spp* in the environment and raw meat products during pig slaughtering, deboning and meat cutting operations
Panoulis C., Genigeorgis C., Kokkinakis M., Tselentis I. (Greece)
- O63 Pork safety and quality through livestock welfare:
1.Welfare of pigs on the farm
Sossidou E.N., Tserveni-Goussi A., Ramantanis S. (Greece)
- O64 Effect of pre-slaughter handling and serology on *salmonella* in pigs
Hamilton D.R., Bobbitt J., Lester S., Pointon A.M. (Australia)

11:00-11:30 Coffee Break

HALL ZEUS B

Resistance

11:30-13:30

Keynote lecture: Emerging antimicrobial resistance in foodborne pathogens

Meng J. (USA)

- O65 Macrolide resistance in porcine streptococci: A human health hazard?
Martel A., Decostere A., Deleener E., Devriese L., Haesebrouck F. (Belgium)
- O66 Adaptive resistance to Biocides and implications of cross-resistance to antimicrobial agents in Foodborne Pathogens
Braoudaki M., Hilton A.C. (UK)
- O67 Tetracycline resistance genes in *Salmonella* from growing pigs and their relationship to antimicrobial use and resistance to other antimicrobials
Bahnsen P.B., Teferedegne B., White B.A. (USA)
- O68 Reported antimicrobial use and *Salmonella* resistance on 90 Alberta swine farms
Rajic A., Deckert A., Reid-Smith R., McFall M., Manninen K., Dewey C., McEwen S. (Canada)
- O69 Vetstat-The Danish Nation-wide monitoring of veterinary medicine use on herd level
Jensen V.F., Jacobsen E., Pharm M.Sc., Wegener H. (Denmark)

SCIENTIFIC PROGRAM

FRIDAY, OCTOBER 3, 2003

- 070 *Salmonella* Typhimurium phage types linked with pigs and their association with human infection in England and Wales
Ward L.R., de Pinna E., Threlfall E.J. (UK)
- 13:30-15:00 Lunch break at the Conference Venue
- 15:00-17:00 Visiting Posters for Topics Intervention measures (PI), Resistance (PR), Slaughtering and Processing of Pork (PS), Pathogenesis (PP)
- 17:00-17:30 Coffee Break
- 17:30-18:30 Poster Discussions for Topic Diagnostics (PD), Pathogenesis (PP)
- 20:15 Departure for Cretan Night

SATURDAY, OCTOBER 4, 2003

HALL ZEUS A

Intervention measures

09:00-11:00

- 071 **Quantification of the spread of *Salmonella* and the effect of 2 feed additives**
Nollet N., Maes D., Dewulf J., Bruggeman G., Molly K., de Zutter L., Van Hoof J., de Kruif A. (Belgium)
- 072 **Implementing a *Salmonella* monitoring programme for pork in Germany**
Blaha Th. (Germany)
- 073 **Slatted pen floors reduce *salmonella* in market swine held in abattoirs**
Hurd H.S., McKean J.D., Gailey J.K., Griffith R.W., O'Connor A.C. (USA)
- 074 **Reduction of *Campylobacter* and *Salmonella* in Pigs treated with a selected Nitrocompound**
Jung Y.S., Anderson R.C., Genovese K.J., Edrington T.S., Callaway T.R., Byrd J.A., Bischoff K.M., Harvey R.B., McReynolds J., Nisbet D.J (USA)
- 075 **Creating an integrated pork safety and quality system in Greece**
Rantsios A. (Greece), Rantsiou K, Cocolin L. (Italy)
- 076 **Reduction of *Salmonella choleraesuis* contamination in pork carcasses by vaccination**
Kolb J., Roof M., Burkhart K. (USA)
- 077 **Reduction of salmonella contamination in pork carcasses by vaccination**
Kolb J., Roof M., Burkhart K. (USA)

SCIENTIFIC PROGRAM

SATURDAY, OCTOBER 4, 2003

- 11:00-11:30** Coffee Break
- 11:30-13:30** Poster discussions for topics Intervention measures (PI), Slaughtering and Processing of Pork (PS)
- 13:30-15:00** Lunch break
- 15:00-16:30** Closing comments, Selection of the site for the next Symposium

HALL ZEUS B

Resistance, Microbial and Parasitic Ecology

09:00-11:00

- O78** Quantifying tetracycline resistance
Glendening C., Mack A., Bowman A., Funk J. (USA)
- O79** Three-year trend in antimicrobial resistance and genotypes among *salmonella* in swine and humans
Gebreyes W., Thakur S., Altier C., Davis R., Wolf L. (USA)
- O80** Antimicrobial agent susceptibility of *campylobacter* and *salmonella* from swine herds with various therapeutic regimens
Guevremont E., Quessy S. (Canada)
- O81** Antimicrobial agents resistance in *campylobacter coli* from swine and humans
Guevremont E., Quessy S. (Canada)
- O82** Faecal shedding of *arcobacter* species in Belgian pigs
Van Driessche E., Houf K., Van Hoof J., De Zutter L. (Belgium)
- O83** Mycobacterial contamination of environment in pig farms in the Czech Republic between 1996 and 2002
Matlova L., Dvorska L., Bartl J., Ayele W.Y., Bartos M., Alexa M., Pavlik I. (Czech Republic)
- O84** Survival of *salmonella* and *escherichia coli* in pig slurry: results of a plot study
Boes J., Alban L., Bagger J., Baggesen D.L., Olsen J.E. (Denmark)
- O85** Control on the illegal use of clenbuterol in pigs in Hong Kong
Sit Th., Tam G. (China)

11:00-11:30 Coffee Break

11:30-13:30 Poster discussions for topics Resistance (PR), Microbial and Parasitic ecology (PMP)

13:30-15:00 Lunch break

15:00-16:30 Closing comments, Selection of the site for the next Symposium

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FOYER HALL ZEUS A & B

Epidemiology

- PE-01 **Epidemiological tools (serotyping, registograms) for *Salmonella* spp. in pork**
Kuri V., Collins M.A., Madden R.H. (UK)
- PE-02 **Association between *Ascaris Suum* and *Salmonella Enterica* in finisher herds**
Boes J., Enoe C. (Denmark)
- PE-03 **Epidemiology of taeniosis taenia solium/cysticercosis infections in Russia**
Bessonov A.S. (Russia)
- PE-04 **DNA fingerprinting of *S.Typhimurium* from a pig longitudinal study**
Clouting C., Liebana E., Davies R.H., Garcia-Migura L., Bedford S. (UK)
- PE-05 **Longitudinal study of *Salmonella enterica* serovar *Typhimurium* infection in three Danish farrow-to-finish swineherds**
Kranker S., Alban L., Boes J. (Denmark)
- PE-06 **Determination of *Toxoplasma GONDII* antibody prevalence in midwest market swine**
McKean J.D., Beary J., Brockus S., O'Connor A.M., Zhou E. (USA)
- PE-07 **The use of a HACCP-based control system in closed pig herds**
Nollet N., Lammertyn K., Houf K., Maes D., de Kruijff A., De Zutter L., Van Hoof J. (Belgium)
- PE-08 **Prevalence of food-borne pathogens of swine from the swine 2001 study**
Bush E., Fedorka-Cray P.J., Gray J., Luchansky J., Wesley I.R., Patton S. (USA)
- PE-09 **USDA multi-agency project: collaboration in animal health, food safety and epidemiology ((CAHFSE)**
Kraeling R.R., Bush E., Bush E.J., Wineland N.E., Anandaraman N., Ladely S.R., Fedorka-Cray P.J (USA)
- PE-10 **Repeated observations on the salmonella culture status of Midwest U.S. herds**
Bahnson P.B., Omran L.M., Fedorka-Cray P.J., Ladely S.R., Troutt H.F. (USA)
- PE-11 ***Salmonella* infection in a multiple-site swine production system in Brazil**
Silva L.E., Gotardi C., Schwarz P., Mostardeiro P., Vizzotto R., Kich J.D., Cardoso M.I. (Brazil)

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Human Health Implications

- PH-01** **Prevalence and number of *salmonella* in retail pork sausages**
Boughton C., Leonard F.C., Egan J., O'Mahony P., Kelly G., Markey B.K., Griffin N. (Ireland)
- PH-02** **The place of pork in the human trichinellosis outbreaks in Russia at the border of the XXIst century**
Ozeretskoyevskaya N.N., Mikhailova L.G., Siskova T.G., Dovgalev A.S., Sergiev V.P. (Russia)

Diagnostics

- PD-01** **Comparison of an excision and a sponge sampling method for measuring salmonella contamination of pig carcasses**
Swanenburg M., Van der Wolf P.J., Urlings H.A.P., Snijders J.M.A. (The Netherlands)
- PD-02** **Evaluation of pooled serum and "meat-juice" in a *Salmonella* ELISA for pig herds**
Davies R.H., Heath P.J., Coxon S.M., Sayers R.A. (UK)
- PD-03** **Real-time PCR assays for the detection of *Mycoplasma hyopneumoniae* in clinical samples**
 Dubosson C.R., Conzelmann C., Zimmermann W., Häni H., Miserez R., Boerlin P., Frey J., Kuhnert P. (Switzerland)
- PD-04** **Use of recombinant ApxIV in serodiagnosis of *Actinobacillus pleuropneumoniae* infections and development of an ApxIV ELISA**
 Dreyfus A., Kuhnert P., Frey J. (Switzerland)
- PD-05** **Stool processing-methods for *salmonella enterica* isolation and PCR detection**
Oliveira C.J.B., Carvalho L.F.O.S., Freschi C.R., Fernandes S.A., Tavechio A.T. (Brazil)
- PD-06** **Comparison of enrichments schemes for the isolation of *yersinia enterocolitica***
 Bowman A., Mack A., Gebreyes W., Funk J. (USA)
- PD-07** **Characterisation of *Salmonella Choleraesuis* by PFGE and Ribotyping**
 Wasyl D., Hoszowski A. (Poland)

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- PD-08 **Comparison of two commercial ELISA for the diagnosis of salmonellosis in swine**
Mejia W., Zapata D., Martin M., Casal J., Mateu E. (Spain)
- PD-09 **Salmonella serology - which samples should be used: Comparison of meatjuice and serum samples of the same pigs**
Leyk W., Seiffert B. (Germany)
- PD-10 **Randomly amplified polymorphic DNA (RAPD) typing of *Salmonella* Senftenberg in animal feed production**
Eriksson J., Lofström C., Rådström P. (Sweden)
- PD-11 **SYBR-Green real time PCR for salmonella detection in meat products**
Croci L., De Medici D., Di Pasquale S., Delibato E., Toti L. (Italy)
- PD-12 **Development of an ELISA test for Salmonella serological monitoring in Brazil**
Kich J.D., Cardoso M., Coldebella A., Piffer I., Vizzotto R., Silva L.E., Castagna S. (Brazil)

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Pathogenesis

- PP-01 **Anti-*Salmonella* lactic acid bacteria from porcine intestinal sources**
Casey P., Casey G., Gardiner G., Tangney M., Stanton C., Ross P., Hill C., Fitzgerald G. (Ireland)

Intervention Measures

- PI-01 **Effects of commercial feed additives on porcine intestinal microflora**
Campbell A.J., Gardiner G.E., Leonard F.C., Lynch P.B., Stanton C., Ross R.P., Lawlor P.G. (Ireland)
- PI-02 **Elimination of *Listeria* from a sausage batter by HHP Treatment**
Farkas J., Andrassy E., Krommer J., László M. (Hungary)
- PI-03 **Failure to prove the effect of feeding on experimental salmonella typhimurium infection in Pigs**
Baggesen D.L., Maribo H., Bodker R. (Denmark)
- PI-04 **Dramatic reductions of in feed medication via immunization against enteric pathogens**
Kolb J., Roof M., Walter D. (Germany)

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- PI-05 **Semi-quantitative risk evaluation for the occurrence of *Salmonella* spec. in swine herds and slaughter plants**
Bla \dot{h} a Th., Austing M.g., Kuehnel K. (Germany)
- PI-06 **Challenges in prevention of trichinellosis in Austria**
Paulsen P., Winkelmayr R., Smulders F.J.M. (Austria)

Slaughtering/Processing/Handling

- PS-01 ***Salmonella* typhimurium carriage at slaughter after an enterocolitis outbreak in a swine herd**
Oliveira C.J.B.O., Carvalho L.F.O.S., Domingues Jr. F.J., Fernandes S.A., Tavechio A.T (Brazil)
- PS-02 **Pork safety and quality through livestock welfare: 2. Pig welfare during pre-slaughter and stunning**
Ramantanis S., Sossidou E.N., Tserveni-Goussi A. (Greece)
- PS-03 ***Salmonella enterica* in pork: Prevalence in the environment carcasses and by-products in the slaughterhouse of a vertically integrated company (2001-2002)**
Stathopoulou E., Genigeorgis C., Panoulis C. (Greece)
- PS-04 **Microbiological and chemical quality of minced meat packaged in modified atmosphere at +1-2°C**
Urso R., Cocolin L., Rantsiou K., Cattaneo P., Comi G. (Italy)
- PS-05 ***Salmonella* isolated in pigs at slaughter and pork products in Brazil**
Bandeira R.M., Castagna S.M.F., Nadvorny A., Schwarz P., da Costa M., Cardoso M.R.I. (Brazil)

Resistance

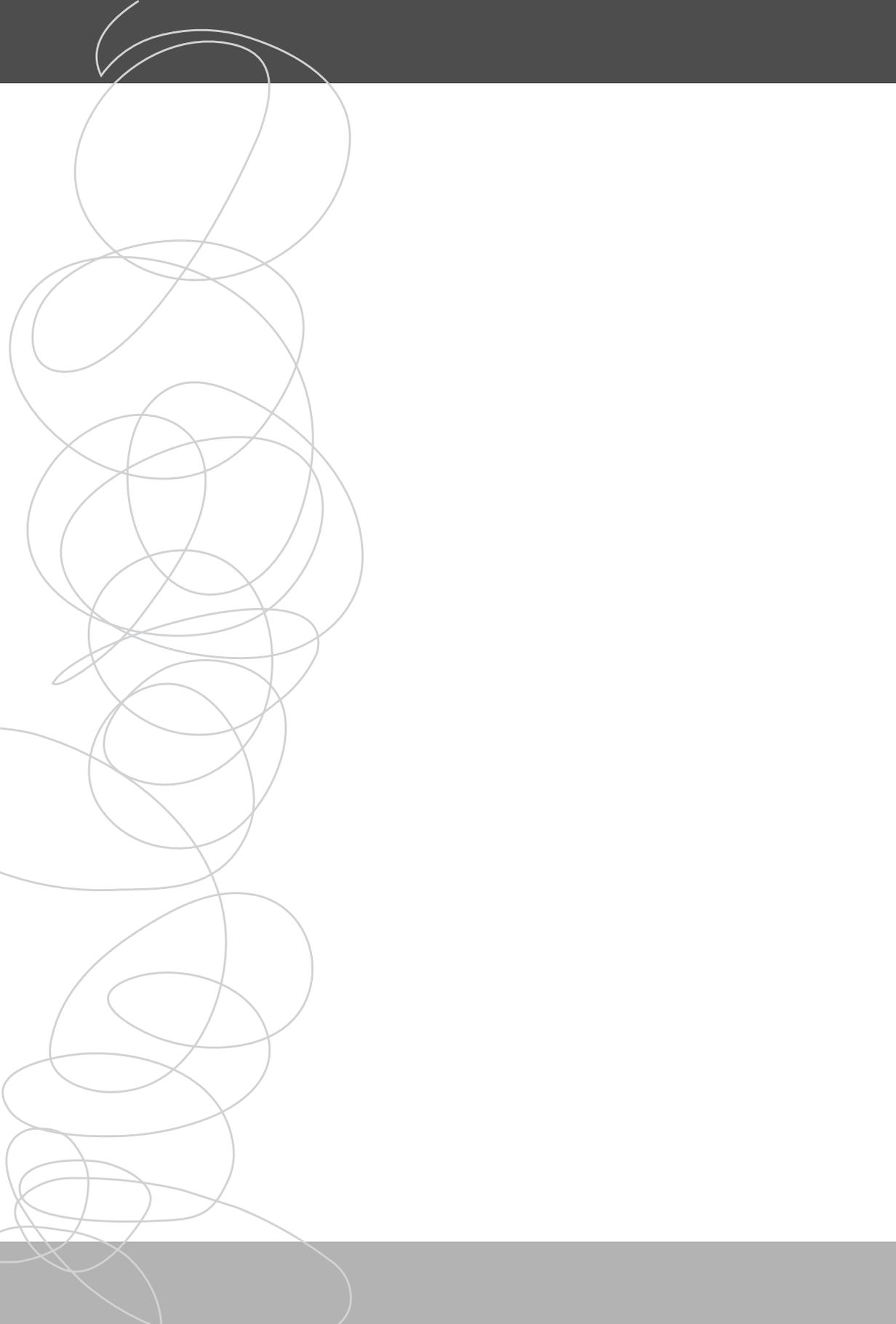
- PR-01 **Mechanisms of resistance in *Salmonella enterica***
Braoudaki M., Hilton A.C. (UK)
- PR-02 **Antimicrobial resistance profile and genetic diversity of *Salmonella enterica* serotypes Typhimurium and Muenchen**
Thakur S., Leaky C., Gebreyes W. (USA)
- PR-03 **Antimicrobial susceptibility of *salmonella* isolated pig carriers**
Mejia W., Zapata D., De Frutos C., Martin M., Casal J., Mateu E., (Spain)
- PR-04 **Effect of chlortetracycline on *Salmonella* and the fecal flora of swine**
Funk J., Mack A., Bowman A., Lejeune J., Wittum T. (USA)

FRIDAY, OCTOBER 3, 2003

- PR-05 **Antimicrobial resistance of *Salmonella* isolates from swine**
 Carvalho L.F.O.S., Oliveira C.J.B., Fernandes S.A.,
 Tavechio A.T (Brazil)
- PR-06 **Antimicrobial resistance of *Salmonella* strains isolated from pork products**
 Castagna S.M.F., Bandeira R.M., Schwarz P.,
 Nadvorny A., Canal C.W., Costa M., Cardoso M.R.I.(Brazil)
- PR-07 **Effect of Tiamulin Clortetracyclin in Health Status and Production in Farm Pigs**
 Radojicic B., Ivetic V., Arsenovic Z. (Serbia)
- PR-08 **Antibiotic resistance of *Salmonella* strains isolated from pig slurry**
 Schmidt V., Cardoso R.I.M. (Brazil)
- PR-09 **Resistance to apramycin of *Salmonella* on *E. coli* isolated from swine**
 Magistrali C., Scuota S., Sensi M., Neri M.C., Maresca C. (Italy)

Microbial and Parasitic Ecology

- PMP-01 **Survival of *Salmonella* and *Escherichia Coli* in pig slurry: simulation of decay**
 Boes J., Alban L., Bagger J., Baggesen D.,
 Olsen J. (Denmark)
- PMP-02 **Risk analysis of *Bacillus* Spp. Isolated from cured pork sausages**
 Morea M., Matarante A., Baruzzi F., Abbrescia A. (Italy)
- PMP-03 **Peracute infection of swine with salmonella**
 Griffith R.W., Hurd H.S., McKean J.D., Gailey J.K., Larsen S.T.,
 Harbaugh E.M. (USA)
- PMP-04 **Microbiological study of warm and chilled meat in Hong Kong**
 Tam G. (China)



Industry Perspective on Food Safety -More Questions than Answers

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A Global Issue

Food safety concerns know no global boundaries. Information is disseminated daily, and even hourly via Internet, which describes concerns ranging from *E. coli* 0157:H7 in American beef to *Listeria monocytogenes* in Greek feta cheese. According to the CDC website (2002), an estimated 1.4 million cases of salmonellosis occur annually in the United States with an estimated 500 or more fatal cases each year. Although these numbers are rather staggering, Hedberg (2001) indicated that pork or pork containing foods were implicated as the source for only 3% of salmonellosis outbreaks reported by CDC from 1990-97. Sarwari et al. (2001) indicated that serotypes impacting human illness may not correlate as well as expected to those found in animals. There remains the question of variable virulence between types of *Salmonella* spp., which might cause one to reconsider treating all *Salmonella* as equals. Investigations into virulence of specific strains of *Listeria monocytogenes* and *Campylobacter* are being conducted as well.

Consumers' awareness of food safety concerns has been increased due to media coverage by newspapers, magazines and talk shows. The regulatory agencies have been feverishly regrouping to try to meet consumer concerns in response to dioxin, BSE and food borne illness outbreaks, some of which have resulted in large, highly publicized recalls. In the United States, change began in the mid-nineties with steps toward required implementation of HACCP in meat plants. This stimulated the promulgation of many other regulations, as well as substantially increased the attention and activity of consumer groups on food safety and policy. These consumer groups are now turning their attention to live animal impacts on food safety. In addition, there has been discussion about coordinating the efforts of agencies that address food safety in the United States as they are covered by a complex set of laws, directives, policies, notices and memos across USDA and FDA (USDA-FSIS, 2003). The Food Safety Council of Japan and the Food Safety Authority in the E.U. are further examples of the desire for centralized oversight of food production systems.

The U.S. has mandated zero tolerance for the presence of *Listeria monocytogenes* or *E. coli* H7:0157, whose presence renders the product adulterated from regulatory perspective. Other countries, such as Canada, have chosen to establish action levels for *Listeria monocytogenes* in low risk products (ICMSF, 2001)

How Do Quality and Food Safety Fit in a Business Plan?

A better question might be, how does one have a business plan without considering quality and food safety?

History of Salmonella and Other Pathogen Efforts: Full Circle

Premium Standard Farms began work in preharvest food safety in 1994 just as U.S. researchers were just beginning to examine and evaluate international efforts to reduce *Salmonella* in live hogs, and to work collaboratively to understand methodologies. Efforts were initially focused on applying HACCP principles to farms and feed mills. The steps were charted and flow diagrams completed. As work on the hazard analyses progressed, it became obvious that without clear food safety objectives,

efforts would be fruitless. Also, it was recognized that there simply was not a sufficient understanding of the systems that might impact end product safety. For example, programs implemented in a feed mill could be negated by recontamination in the finisher or by pig to pig contamination in lairage. Given the number of sources for *Salmonella* contamination or recontamination (Wray and Davies, 2003), attempts to reduce in a large system are rather daunting. Surveys have shown farms to be negative, but *Salmonella* isolated from the digestive tract at the slaughterhouse (Boudry, 2002). The mechanisms of keeping farms or entire production systems have not been adequately elucidated or determined to be sustainable.

Because data had not yet been generated to support clear food safety objectives or critical control point in the preharvest sector, efforts were focused on slaughtering practices. Admittedly this decision was driven in part by the USDA-FSIS performance standards and directives. This focus resulted in implementation of technologies such as steam vacuums, carcass pasteurization and intensive visual inspection to comply with these standards. In addition to regulatory concerns, some customers have standards for *Salmonella* on products they purchase. As a result of these interventions, percentages of positive carcasses have been reduced since the initiation of testing as indicated by USDA data (USDA-FSIS, 2000). *Salmonella* prevalence in large plants has declined to 1.7% positive carcasses in swine, a 100 percent compliance rate. Achieving these low levels has allowed reconsideration of preharvest interventions as a possible means to take carcass counts beyond the level they are now. In addition there is still a nagging concern that carcass values may be underestimating the actual product contamination in pork cuts, offal items and trimmings during fabrication and at retail (Duffy et al., 2001; Zerby et al. 1998). It seems logical to work backwards from slaughter toward live pig production through the first step is what is now know as peri-harvest. As a result of research showing the influence of lairage (Hurd, 2001), additional consideration is being given to new controls prior to slaughter.

How Does Industry use Research?

-The Chicken and the Egg...

Production companies must have quality research to facilitate efforts to be proactive regarding food safety risks. Research that is not generated in a well-balanced format may be misused or misinterpreted causing a negative impact on industry. Additionally, it is possible that research may falsely elevate the importance of an issue until it finally becomes a self-fulfilling prophecy. Research must maintain a solid anchor to tangible food safety objectives that are practical and applicable. The validation of intervention strategies across all levels of the food production continuum are a crucial need.

Other forces may cause efforts to be increased in the preharvest area. One example is the focus on the pathogen *E. coli* 0157:H7. What has occurred in the beef industry is a good example of what could occur with any animal borne pathogen. Those companies which grind beef for hamburger and slaughter the cattle are nearly to the point of exhausting the existing arsenal of interventions. This has resulted in increased pressure on the research community as well as on live cattle suppliers to find and implement live animal technologies to reduce the load entering the slaughter facilities. This is a very good example of hurdle technology (Leistner and Rodel, 1976) where efforts must be layered in order to be most effective.

Poultry and egg production, in particular, are also much further along the path of preharvest controls of pathogens due primarily to the concerns associated with *Salmonella* enteritidis. Interventions include competitive exclusion cultures and feed additives. Interestingly, the Danes have specifically excluded competitive exclusion and vaccines from their reduction strategy due to a belief that these might "mask the *Salmonella* problem" (Wegener et. al. 2003)

So why is pork trailing the preharvest progress of these two species with their pathogens of concern? It is primarily due to the lack of a triggering event such as those experienced by other species. Additionally,

pork still does not have clearly established relationships between on farm and product levels of *Salmonella*. However, the identification of antibiotic resistant pathogens such as multiple drug resistant *Salmonella* typhimurim DT104 may serve as an additional catalyst to reduce *Salmonella* levels in swine.

There is also a strong need for researchers to guide industry on testing methodologies, yet it is difficult for researchers to agree on the definitive methods that will represent live pigs, their environment and pork products. Researchers generally use lymph nodes or carcass values as a means to assess post-harvest implications, but these may not be sufficient. Maddox (2003) describes the large number of methodologies that were identified during her search for detection methods. Methodologies that industry can use to track *Salmonella* levels must be rapid, accurate and cost effective.

Why is PSF putting An Effort in Verification and Quality?

Companies around the world are recognizing that they it is impossible to be competitive merely by marketing a commodity such as pork. What started as areas for specialized companies to differentiate themselves has turned into a race to raise the bar and establish the next niche. Once that is accomplished, the competition quickly adopts the same technologies or programs and the hunt is on for the next way to differentiate product in the marketplace. This is driven by demands from export customers as well as by some domestic customers seeking to distinguish themselves from retail giants like Wal-Mart. The U.K. led the way in these efforts with its welfare-friendly and high-palatability programs marketed at stores like Marks and Spencer, and Sainsbury's.

Who's Going to Pay for Food Safety?

Unfortunately most customers are unwilling to pay more for the same product with new food safety enhancements. With rare exceptions such as irradiation, they view these upgrades as a cost of doing business to their supplier. However, as interventions are implemented which add cost, those costs will have to be covered by either revenues or benefits such as improved production or yields. If this does not occur, it becomes an additional component of the cost of the product and is passed on more discreetly through the system. The problem with the latter solution is that unless all companies are forced to implement interventions, costs between them are no longer competitive, penalizing the company attempting to "do right".

Food safety is something that the end consumer understandably views as a right. Those of us in the business of producing food know it is something not to be taken for granted and that there are many steps through the chain of production that will help us control the risks. Objective measurements or performance standards are needed as an incentive for compliance, and to tie live animal efforts to final-product safety.

According to Wegener et al. (2003), the Danes have saved U.S. \$25.5 million in costs to society by controlling *Salmonella* in multiple species. This cost their pork industry almost U.S. \$.08/kg so it is very important that the link between that very real cost and the calculated benefit for society is correct. An additional reason the Danish program has been successful is that it was done on a national scope causing costs to be incurred across all producers.

Answering the Questions: What Does Business Need from the Research Community?

- Evidence of a more concrete relationship between live animal and product levels of pathogens.
- Evidence of the relationship between product levels of pathogens and human illness.
- A systematic evaluation of interventions that are correlated with end product results.
- Cost-effective interventions proven to impact products.
- Validation of existing and new interventions conducted on the farms and in the plants.
- Uniform testing methodology and interpretation of live animals, carcass and finished products.
- A recommendation for a uniform control plan and means to measure results.

- Continued research into the relevance of emerging pathogens (e.g. toxoplasmosis).
- The evaluation of control programs for *Trichina* and *Salmonella* as templates for reduction of other pathogens.

What Will Define "Safe Pork" in the Future?

Besides the current pathogens of concern, other critical issues may be involved in defining a safe product in the future. These will undoubtedly include the continued focus on antibiotic resistant pathogens and GMO concerns. However, non-traditional components of food production such as product traceability are increasing in consumer importance. Social issues such as antibiotic usage, animal welfare and the environment are being used by consumers to determine their purchasing choices. Researchers and industry must work cooperatively to be proactive in addressing these issues and building consumer confidence in our products.

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Evaluation of diagnostic methods used in zoonosis-surveillance programs

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Summary: The purpose of the present work is to set focus on the importance of built-in validation of diagnostic methods in zoonoses-surveillance programs. Appropriate and ongoing evaluation schemes are preconditions for estimating true prevalences of zoonotic infections or infestations. Estimates of true prevalences are crucial for optimizing surveillance programs so they are both cost effective and provide the best possible tool for assessing consumer safety. Using 3 examples we illustrate some of the diagnostic challenges in zoonosis surveillance that potentially could be met by appropriate validation schemes and knowledge about the performance of the diagnostic methods. We recommend that estimates of within and between laboratory variation, analytical and diagnostic sensitivity and specificity are made accessible in the public domain as part of a quality-assurance system for diagnostic methods in surveillance programs. Furthermore, we recommend that diagnostic methods be subject to an ongoing validation in any surveillance program.

Keywords: Consumer safety, Diagnostic performance, True prevalence, Built-in validation, Latent-class methodology

Introduction: Worldwide there has been an increased awareness and public concern over food-borne pathogens over the past two decades. To a large extent this has been due to a number of serious human outbreaks caused by e.g. *Salmonella*, *Campylobacter* and *Escherichia coli* O157:H7 (Nielsen, 2002). In 2001, salmonellosis and *campylobacteriosis* were by far the most frequently reported zoonoses in the European Union with approximately 159,000 human cases each (Anon., 2003a). In addition, the emergence of new zoonoses such as the BSE related variant Creutzfeld-Jakobs disease has enforced the focus on food safety issues. Veterinary surveillance activities in many countries have improved in the same period. This was due partly to a need for better information on the disease status of the national herd in order to support livestock industries and partly due to improved epidemiological methods and implementation of computerized databases. This development has been stimulated by legislation, which governs international trade of animals and animal products and by the concern over food-borne pathogens and antimicrobial drug resistance (Gibbens and Wilesmith, 2000). Although the majority of food-borne diseases most likely are not attributable to pork (Nielsen, 2002), food safety has been a driving incentive for the pork industry in Denmark, giving a high priority to monitoring and surveillance of zoonotic infections in swine. A well-known example is the nation-wide *Salmonella enterica* surveillance and control program in Danish finishing herds (Mousing et al., 1997; Nielsen et al., 2001). In 2002, the expenses on the surveillance of *Salmonella* amounted to Euro 6.9 million (Nielsen and Korsgaard, 2003).

Within the European Union each member state has to collect epidemiological data on zoonoses to comply with the community strategy. All member states are at present contributing to an annual report (Anon., 2003a) on trends and sources of zoonotic agents according to article 5 of Directive 92/117/EEC, which contains information on the situation regarding zoonoses in animals, feedstuffs, food and man. However, the quality of the data suffers from un-harmonized surveillance systems, which makes it very difficult to draw inferences on the trends of the prevalences of zoonotic agents within the community. To overcome the need for harmonized and valid zoonosis-surveillance programs in the European Union and worldwide, it is of utmost importance to document the diagnostic processes involved. Knowledge of diagnostic test characteristics such as diagnostic sensitivity and specificity

is a precondition to obtain reliable estimates of true prevalence. In turn, true prevalence estimates are crucial for optimizing surveillance programs so they are both cost effective and provide the best possible tool for ensuring a high consumer safety. Therefore, an ongoing validation process is needed for the diagnostic methods used in surveillance programs, in order to detect possible changes with time in disease prevalence and diagnostic performance.

We have illustrated a few of the diagnostic challenges in zoonosis surveillance using data from screening, surveillance and monitoring programs of *Salmonella*, *Campylobacter* and verotoxin producing *Escherichia coli* in Denmark.

Example 1, Salmonella surveillance: In the Danish *Salmonella* surveillance and control program, finisher herds are monitored for the presence of *Salmonella*-specific antibodies in meat juice. Based on the serological results, all finisher herds are assigned to 3 different herd levels on a monthly basis. At the same time, a national average of seropositive samples is calculated. This average is used as a measure for the general level of seropositive finishers in Denmark. Similarly, fresh pork is monitored for *S. enterica* at the abattoirs every month. From 1993 to 2000, the surveillance was conducted by bacteriological examination of different pork cuts. From 2001 and onwards, *Salmonella* testing on pig meat has been based on swabbing of carcasses. This latter method is twice as sensitive as the one previously used (S. rensen et al. 2001). The apparent *Salmonella* prevalence in pork was adjusted accordingly to allow for comparisons (Figure 1).

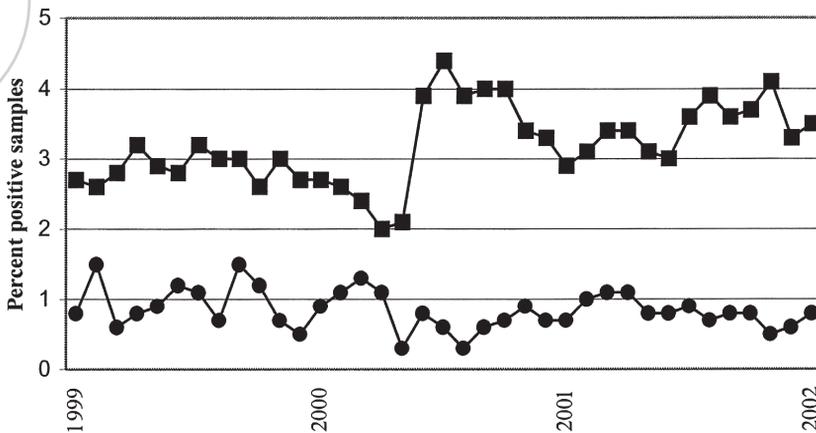


Figure 1. The prevalence of *Salmonella*-positive samples assessed by meat-juice ELISA (- -) and bacteriological examination of pork cuts (- -).

The prevalence of culture-positive samples has declined from 3-4 % in 1993 to 0.7 % in 2000, and has remained at a similar low level since. In contrast, in June 2000, an unusual and unexpected increase in the number of seropositive samples was observed in the course of just 2 weeks. The prevalence of seropositive samples increased from 2 % to 4 %, and remained at higher level for more than a year. However, at the same time the prevalence of *Salmonella* in pork was lower than usual. These observations were unexpected, as several earlier Danish investigations had demonstrated a clear correlation between the prevalence of seropositive and bacteriological positive samples on herd level. A large investigation was initiated in order to clarify, whether the observed increase in seropositive samples was due to a true increase in *Salmonella* load in the finishers, or whether the increase was due to a drift in the mix-ELISA (Nielsen et al., 1995). The mix-ELISA is routinely calibrated by use of 8 control

sera on all plates. Additionally, the mix-ELISA is tested on a monthly basis against a panel of 40 other field sera from swine in order to ensure that the results of the ordinary control sera do not drift over time. No deviations in the daily control sera or monthly calibration sera were observed. For comparison, results from the national *Salmonella* surveillance of all breeder and multiplier herds were studied too. In these herds, 10 gilts are blood sampled every month and tested for *Salmonella* antibodies. Half of the herds also produce finishers for slaughter, which are tested for *Salmonella* antibodies as mentioned earlier. In the breeder and multiplier herds, the seroprevalence based on serum samples from gilts remained at a steady level throughout 2000, while the seroprevalence based on meat-juice samples from their finishers increased as was observed in ordinary finisher herds. No satisfactory explanations could be offered for these observations. A similar scenario has not been observed since.

Example 2, *Campylobacter* monitoring and screening: Samples of intestinal contents are collected routinely from cattle, pigs and poultry at different abattoirs throughout the country, as part of the Danish Antimicrobial Resistance Monitoring Programme (DANMAP). According to the DANMAP procedure, a single caecal sample per herd is collected from 200 to 250 randomly chosen pig herds each year. Samples are analyzed for *Campylobacter* species according to standard bacteriological procedures, followed by determination of antimicrobial resistance profiles. The result – the prevalence of samples positive for *Campylobacter* – thus is an estimate at both pig and herd level. The DANMAP results for *Campylobacter* from 1997 to 2002 are shown in Table 1.

| Study | Year | Number of herds tested | <i>Campylobacter</i> positive caecal samples (%) | | |
|--------|------|------------------------|--|----------------|------------------|
| | | | <i>Campylobacter</i> spp. | <i>C. coli</i> | <i>C. jejuni</i> |
| DANMAP | 1997 | 245 | 56 | 53 | 2.4 |
| | 1998 | 194 | 60 | 52 | 6.2 |
| | 1999 | 244 | 46 | 41 | 4.5 |
| | 2000 | 277 | 60 | 56 | 4.0 |
| | 2001 | 238 | 77 | 69 | 2.9 |
| | 2002 | 240 | 80 | 79 | 1.6 |
| DBMC | 2002 | 247 | 92 | 90 | 2.3 |

Table 1. Percent *Campylobacter* positive caecal samples from swine.

Two observations can be made: (1) the apparent prevalence of *Campylobacter* species (including non-typed isolates) using the DANMAP method ranges from 46-80 % and has been increasing over the past few years; (2) only the prevalence of *C. coli* has increased (from 41 % to 79 %), while *C. jejuni* in pigs has remained at a relatively constant, low level (1.6-6.2 %). In 2002, the Danish Bacon & Meat Council (DBMC) carried out a screening for *C. jejuni* in pigs at several abattoirs. Caecal samples from 247 herds were examined, and per herd 5 pigs were sampled. Samples were analyzed according to the same bacteriological procedures as in the DANMAP surveys. The results of the screening are summarized in Table 1 (see also Boes et al., 2003, in this issue). All 247 herds were positive for *Campylobacter* species (herd prevalence = 100 %), and 92 % of pigs were *Campylobacter* positive. Pig prevalences of *C. coli* and *C. jejuni* positive caecal samples were 90.1 % and 2.3 %, respectively. In the DANMAP study, taking 1 sample per pig herd resulted in quite low *Campylobacter* prevalences (especially *C. coli*), whereas in the DBMC study, herd sensitivity was improved by taking 5 samples per herd, resulting in higher prevalences of positive findings. It should be added that in each of 247

herds in the DBMC screening *Campylobacter* was detected in at least 2 pigs, and usually all 5 pigs sampled per herd were positive. Samples collected in the DBMC screening were analyzed in the laboratory within 24 hours after collection. In contrast, DANMAP samples typically were accumulated over a one-week period, and then analyzed. It might be speculated that this procedure was detrimental to at least a proportion of samples containing *Campylobacter*. Recently, the DANMAP procedure was changed so that samples collected in the DANMAP survey are analyzed more frequently, which could explain the increase in *Campylobacter* prevalence in recent years. Interestingly, the negative effect of storing samples for up to 1 week is more pronounced for *C. coli* than for *C. jejuni*, as the low apparent *C. jejuni* prevalence is comparable between DANMAP and the DBMC study.

Example 3, Detection of verotoxin producing *Escherichia coli*: Verotoxin producing *E. coli* (VTEC), like O157:H7 known from ruminants, traditionally has not been related to swine and pork. However, verotoxin producing *E. coli* O157:H7 have recently been detected in swine in several countries (Table 2).

| Country | Prevalence of culture-positive faecal samples (%) |
|-------------|---|
| Chile | 69 |
| Japan | 1.4 |
| Netherlands | 0.7 |
| Norway | 0.1 |
| UK | 0.16 |
| USA | 3.6 |

Table 2. Prevalence of verotoxigenic *E. coli* O157:H7 in swine faeces.

Additionally, an experimental inoculation study (Cornick and Helgersen, 2003), demonstrated that swine readily become colonized by *E. coli* O157:H7, and that colonized pigs transmit the infection to naive pen mates. The veterinary and public health focus has so far mainly been on O157:H7, but this strategy might need to be reconsidered. Increasing evidence shows that the virulence cassette of the *eae* gene in combination with the *vtx2* gene – rather than the serotype – is associated with haemolytic uraemic syndrome (HUS) in children and haemorrhagic colitis in adults. Most veterinary and human diagnostic laboratories traditionally only examine for O157:H7 or few other of the most common VTEC serotypes, as detection of all VTEC types demands colony hybridization with probes for verotoxin and *eae* genes. As a consequence, the non-O157 VTEC are most likely underreported. In a US study (Keen et al., 2003), 1,102 healthy swine were examined for VTEC O157, O111 and O26 while the animals were on display on fairs during 2002. Pigs were found to harbor all 3 VTEC serotypes. At present very little is known about the general occurrence of VTEC in swine and pork worldwide. Future prevalence studies should employ colony hybridization with probes for verotoxin- and *eae* genes in contrast to slide agglutination for just O157:H7. Until a broad VTEC screening for different VTEC types in swine and pork is conducted, it remains difficult to estimate the human risk for pork related VTEC infections. The difference between slide agglutination for O157 and colony hybridization can be illustrated by an example from Denmark, where human cases of gastroenteritis are routinely examined for a number of zoonotic agents, including VTEC. Of the 14 counties in Denmark, 7 counties perform slide agglutination for O157:H7 and a few other common VTEC serotypes. The remaining counties submit stool samples to the central Danish diagnostic laboratory, where examination includes colony hybridization using probes for verotoxin- and *eae* genes prior to slide agglutination. In counties using just slide agglutination the incidence is < 1-2 cases per 100,000 inhabitants per year whereas in counties using the central laboratory VTEC incidence is 3-6 cases per 100,000 inhabitants per year (Anon, 2003b).

Discussion: In the surveillance of *Salmonella* ongoing double-classification of samples from different entities of herds followed by statistical estimation of diagnostic sensitivity and specificity and true

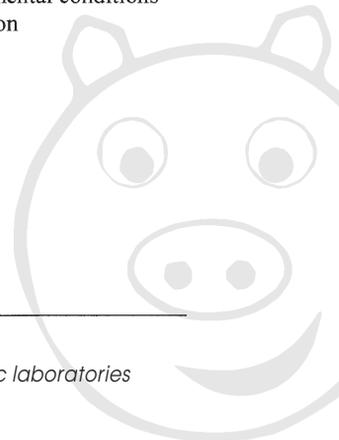
prevalences could potentially have resolved the question whether the increase in seroprevalence in 2000 was real or merely a result of drifts in the diagnostic methods. This could have been accomplished applying latent-class methods (En_e et al., 2000). This approach is possible when 2 or more diagnostic tests are available and applicable. Even if they are far from being perfect they can still be used in the surveillance and will yield valid estimates of the true prevalence. Latent-class methods have been used for validation of the mix-ELISA for detection of *Salmonella*-specific antibodies in meat juice (En_e et al., 2001). Crude estimates of apparent prevalences can be highly biased when compared to estimated true prevalences, potentially leading to serious underreporting (En_e et al., 2003). In the monitoring of *Campylobacter* in DANMAP the importance of common criteria for data collection becomes evident when prevalences of culture positive samples are compared with the results from the DBMC study. Thus a sampling scheme based on 5 caecal samples per herd instead of 1 improves the herd-level sensitivity significantly. A direct comparison of the prevalences from these 2 studies without taking sampling strategy into account will lead to mistakes in inference. It is important to realize that the diagnostic performance of aggregate testing to a large extent depends on the number of samples taken. Thus, uniform sampling strategies including appropriate handling and storing of samples prior to testing and between-laboratory testing could have increased the diagnostic precision and made inference easier. The importance of standardization of diagnostic processes is further illustrated by the differences in incidence of human VTEC infections due to different diagnostic strategies in counties in Denmark.

In the European Union surveillance systems are primarily based on existing programs in member states. However, the community strategy implies common criteria for data collection. Moreover, member states shall ensure that diagnostic laboratories apply quality-assurance systems, which conform to the requirements of Standard EN/ISO 17025 (Table 3).

| Management | Technical |
|--|--|
| Organization | General requirements |
| Quality system | Personnel |
| Document control | Accommodation and environmental conditions |
| Reviews of requests, tenders and contracts | Test methods and test validation |
| Subcontracting | Equipment |
| Purchasing | Measurement traceability |
| Service to clients | Sampling |
| Complaints | Handling of test samples |
| Control of non-conforming tests | Assuring quality of results |
| Corrective actions | Reporting results |
| Preventive actions | |
| Control of records | |
| Internal audits | |
| Management reviews | |

Table 3. Elements of the standard EN/ISO17025 standard for diagnostic laboratories (from Gajadhar and Forbes, 2002).

The importance of implementing such systems is illustrated in an excellent way by Gajadhar and Forbes, (2002), who describe an internationally recognized quality-assurance system for diagnostic parasitology, with example data on trichinellosis. Equivalent quality-assurance systems should be set up, if not already in place, for the other important zoonoses in pork and preferably published in peer-reviewed international journals. As part of the quality assurance, diagnostic laboratories should regularly participate in collaborative testing organized or coordinated by the national reference laboratory such as the first international ring trial of ELISAs for *Salmonella*-antibody detection in swine (van der



Heijden, 2001). Basic information gathered in compliance with the ISO/IEC Standard 17025 should be made public e.g. on the Internet or other public domains because it is an absolute precondition for optimizing surveillance or monitoring programs and drawing the right inference from the results. Thus, estimates of diagnostic sensitivity and specificity are needed in order to estimate true prevalences of disease; measures of precision and accuracy are needed for appropriately optimizing diagnostic methods e.g. reduce the number of repeated testing on the same specimen (Ekeröth et al., 2003); ongoing validation (estimates of diagnostic sensitivity and specificity) of diagnostic methods is necessary to assess the possible change of true prevalence with time; innate test characteristics such as analytical sensitivity and specificity are needed to make proper selection of the most appropriate diagnostic measure.

Recommendations and conclusions: To summarize, we recommend that an effort should be made to apply common criteria for data collection. Furthermore, quality-assurance systems that conform to the requirements of ISO/IEC Standard 17025 should be implemented. As a part of the quality-assurance system, basic information should be made available on the Internet or other public domains covering as a minimum requirement:

1. Precision - measured as the variation of repeated analyses on a specimen.
2. Accuracy - measured through ongoing validation and estimates of changes in performance with time and through ring trials.
3. Estimates of analytical sensitivity and specificity.
4. Estimates of sensitivity and specificity or any other relevant measure of validity such as likelihood ratio, assessed in the population or comparable to the population in which the diagnostic method is going to be used.

All this information should be the result of an ongoing validation scheme, as it is evident that the validity of diagnostic methods very likely changes with time. An ongoing validation of disease measures is costly and laborious. However, the latent-class approach (Enøe et al., 2000) may be a valuable contribution to make ongoing validation economically feasible. Latent-class methods should be incorporated in surveillance programs and disease monitoring systems whenever possible in order to provide an ongoing and dynamic validation of diagnostic processes.

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Ensuring the safety of animal feed

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Summary: A general outline is presented of measures for the production of safe animal feed. This is based on the setting of so-called 'feed safety objectives' which make use of principles that relate to animal health, animal welfare, legal aspects of farm practices and human food safety objectives for products of animal origin. Particular emphasis will be put on the types of feed used in relation to feedborne animal diseases caused by infectious and chemical agents and on the relationship between animal feed and zoonotic foodborne diseases. In addition the influence of feed on animal welfare will be discussed. To produce safe animal feed, a pro-active control system is advocated. This approach has been very successful in relation to human food and involves the use of 'good manufacturing practices' (GMP) and the 'hazard analysis critical control point' (HACCP) concept as the main tools. However, it has been shown that the HACCP-system has certain shortcomings. To counteract these shortcomings, product traceability and hazard early-warning systems have been developed and will also be presented.

Keywords: Feed, health, welfare, environment, legislation, food safety

Introduction: Feedstuffs play an important role in maintaining the health of production animals and therefore of humans. In relation to food safety, the slogan 'healthy animals, healthy humans' is often used to demonstrate the clear relationship that exists between the health status of animals and that of human beings. Experience has shown that the transmission of diseases from domestic animals to man can only be prevented effectively by improving the health care of the animals themselves. It is even more of a challenge to prevent the transmission of zoonotic agents because, as the human population has increased, there has been a concomitant increase in the number of production animals. Factors involved in disease control include the availability of safe feedstuffs, husbandry practices, immunisation and the use of antimicrobials and other veterinary drugs. Strategies that have been explored to control foodborne human pathogens include the administration of selected microbial cultures to piglets and day-old chicks in order to establish a balanced gut microflora and increase colonisation resistance. In the case of ruminants, attempts have been made to reduce carriage of *Escherichia coli* O157 by using special dietary formulations. However, neither of these approaches to gut flora manipulation has been entirely successful.

In this overview, a general outline is presented of measures for the production of safe animal feed. This is based on the setting of so-called 'feed safety objectives' which make use of principles that relate to animal health, animal welfare, legal aspects of farm practices and human food safety objectives for products of animal origin.

1. General outline: A general outline of an approach to the production of safe animal feed is presented in Figure 1. The system is based on the setting of 'feed safety objectives', which make use of principles that relate to animal health and welfare and environmental and legal aspects, as well as the safety criteria set for human foods of animal origin, the so-called 'food safety objectives'. To set feed safety objectives, the principles of risk analysis must be used.

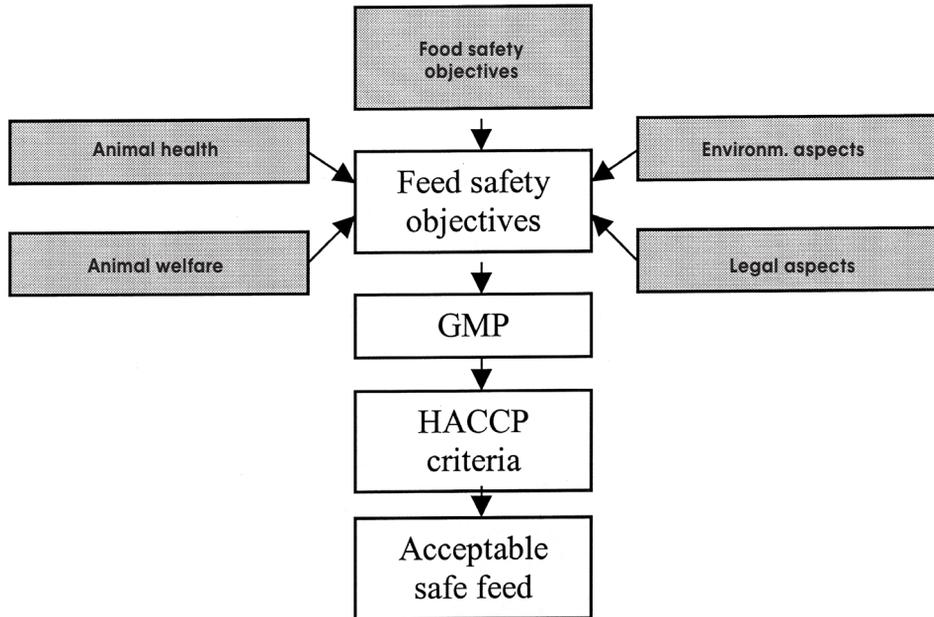


Figure 1. General outline for the production of safe animal feed

To meet the feed safety objectives, a proactive control system based on scientific data is advocated. This approach has been very successful in relation to human food and involves the use of 'good manufacturing practices' (GMP) and the 'hazard analysis critical control point' (HACCP) concept as the main tools.

2. Feed safety objectives: The starting point is the food safety objectives for chemical and microbiological contaminants, including zoonotic disease agents, which are essential to produce acceptable, safe food products for human consumption. For both types of hazards, a 'transfer factor' needs to be taken into account (Figure 2). In passing through the intestinal tract, unwanted chemical substances may become diluted, decomposed, etc. In the case of infectious zoonotic agents, an increase in numbers is possible. These phenomena should be considered in setting feed safety objectives.

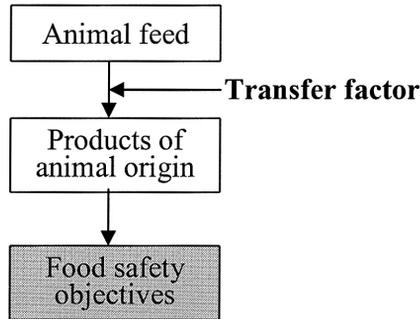


Figure 2. The significance of the transfer factor in setting feed safety objectives.

3. Animal diseases, welfare and other aspects: Feed should not contain infectious agents or toxic substances that can cause disease in animals. Examples include the virus responsible for Foot and Mouth disease and *Bacillus anthracis*, the causative agent of anthrax. As far as toxic and deleterious components are concerned, naturally occurring constituents of animal feed such as plant toxins and extraneous contaminants e.g. heavy metals and dioxins may be present from time to time. Also the suitability of the feed is an important part of animal welfare. Feed must be appropriate to the animal being fed, available in sufficient quantities to satisfy demand at all stages of animal production and be readily accessible to all individuals. Finally, animal feed needs to meet certain legal and other requirements. These relate to zoonotic diseases, trade protection, environmental aspects, etc.

4. Feedborne animal diseases: Feed ingredients are regularly subject to contamination from diverse sources, including environmental pollution and the activities of insects and micro-organisms. This is in addition to endogenous toxins associated with some of the plant materials used. Many of these contaminants cause adverse health effects such as infections and intoxications in the animals and may be transmissible to humans.

4.1 Infectious diseases. There are many infectious disease agents which are transmitted by feed to animals. Some of them are discussed briefly here as examples and an indication given of their control by the application of GMP and HACCP in the production process.

Foot and Mouth Disease (FMD). FMD is a devastating disease of livestock and is characterized by fever and blister-like lesions on the tongue and lips, in the mouth, on the teats, and among the feet. All species of cloven-hoofed animals are susceptible and the disease is extremely contagious. FMD is endemic in many areas of the world, including several countries in Africa, as well as in Asia and South America. In 2001, the disease was confirmed in the United Kingdom, France, The Netherlands, the Republic of Ireland, Argentina and Uruguay. The United Kingdom reported 2030 cases of FMD, slaughtered almost 4 million animals, and took 7 months to control the disease outbreak. Financial losses as a result of FMD can be considerable. There are direct losses due to deaths in young animals, loss of milk and meat and a decrease in growth performance. The costs associated with eradication or control can be high and, in addition, there are indirect losses due to the imposition of trade restrictions. The disease is caused by an Aphthovirus member of the family Picornaviridae and is not a threat to public health.

The virus can easily be controlled by immunisation. Due to its extremely contagious character control of the virus by other means, such as bio-security, is difficult. FMD virus can survive for long periods of time in dark and moist conditions. Therefore non-processed feed is considered to be an important route for transmission of the virus. The virus is easily killed by mild heat treatment, and is completely

inactivated during pasteurisation at 75 oC for a few seconds. The FMD virus is also extremely sensitive to pH. At pH values above 9 and below 6 the virus is rapidly destroyed. For this reason, acidification of feed either by fermentation or adding e.g. citric acid, is effective.

Trichinellosis. Trichinellosis is caused by the nematode *Trichinella spiralis* which parasitises the intestinal tract of mammals, particularly pigs. The larvae encyst in the tissues, particularly the muscles which act as a source of infection for humans who consume raw or partially cooked meat. The clinical manifestations include fever, muscle pain, encephalitis and myocarditis. Death occurs only rarely. The cysts can be transmitted by non-processed feed. Treatments like freezing at -18 oC for 20 days or heat treatment cause inactivation. Effective cooking of raw meat and table scraps before feeding to farm animals will eliminate transmission. This is also the case when traditional rendering temperatures are used.

Bovine Spongiform Encephalopathy (BSE). BSE is a disease of cattle which was first recognised in Great Britain in 1986. Cases have since occurred in many other countries. It is believed now that the disease is caused by a natural protein which folds in the wrong way and then causes other similar proteins to adopt a similar shape. The new form gradually accumulates and spreads. It can transmit disease from animal to animal at least experimentally and from contaminated animal tissue to humans (Bruce et al., 1997a; Bruce et al., 1997b). The complete exclusion of mammalian meat and bone meal (MBM) from all farm animal feed since 1988 resulted in a gradual decrease in the incidence rate of BSE. Since the disease has an incubation period of about 5 years, it was expected that action taken in 1988-90 would take some years to show through. Studies revealed that traditional rendering of animal offal contaminated with BSE prions was not sufficient to inactivate their infectivity in test animals (Fraser et al., 1988). Therefore, the European Commission decided in 1993 to use a higher standard for the treatment of animal waste (133 oC, 3 bars of pressure for twenty minutes). In 1994, the EU Commission also introduced a ban on the feeding of mammalian meat and bone meal to cattle, sheep and goats.

4.2 Mycotoxicoses. Many mycotoxins are harmful when consumed by animals. The toxins can accumulate in maturing corn, cereals, soybeans, sorghum, peanuts, and other feed crops in the field and in grain during transportation (Christensen, 1982). The toxins may be produced during storage under conditions favourable for the growth of the toxin-producing fungus or fungi. The effects in domestic animals include allergic reactions, reproductive failure, unthriftiness, loss of appetite, feed refusal, suppression of the immune system, decreased feed efficiency, and mortality (Hesseltine and Mehlman, 1977) (Table 1).

Table 1. Major mycotoxins, feed products affected and possible effects on animals (source: [myhttp://www.aces.edu/department/grain/ANR767.htm](http://www.aces.edu/department/grain/ANR767.htm))

| Mycotoxins | Feeds affected | Possible effects on animals |
|---------------------------|----------------------------------|--|
| <i>Aspergillus</i> toxins | | |
| Aflatoxins | Cereal grains, peanuts, soybeans | Hepatotoxic, carcinogenic, reduced growth rate, hemorrhagic enteritis, suppression of immunity to infection and decreased productivity |
| Ochratoxins | Cereal grains | Toxic to kidneys and liver, abortion, poor feed conversion, reduced growth rate and reduced immunity to infections. |
| Sterigmatocystin | Cereal grains | Toxic and carcinogenic. |
| Tremorgenic toxin | Cereal grains, peanuts, soybeans | Tremors and convulsions. |

KEYNOTE LECTURES

| <i>Penicillium toxins</i> | | |
|---------------------------|-------------------------------|--|
| Patulin | Cereal grains, apple products | Haemorrhages of lung and brain, edema and toxic to kidneys. |
| <i>Fusarium toxins</i> | | |
| Zearalone | Cereal grains | Hyperestrogenism, infertility, stunting and even death. |
| Deoxynivalenol | Cereal grains | Feed refusal by swine, cats and dogs, reduction in weight gain. |
| Trichotecenes | Cereal grains | Severe inflammation of gastrointestinal tract and possible haemorrhage, oedema, vomiting and diarrhoea; infertility, degeneration of bone marrow, slow growth and sterility. |
| Fumonisin | Corn | Leucoencephalomalacia in horses. |
| Ergot toxins | Cereal grains | Vasoconstriction and loss of extremities (tails, ears, feet, etc). |
| Ergovaline | Fescue | Abortion and reduced weight gain. |

Three genera of fungi - *Aspergillus*, *Penicillium* and *Fusarium* - are the ones involved most frequently in cases of mycotoxin contamination in corn, small grains, and soybeans (Table 1).

Pelletizing feeds may eliminate fungi present in the stock but will not reduce or eliminate aflatoxin present in any of the ingredients. Recently, the addition of binding agents such as hydrated sodium or calcium aluminosilicate and bentonite clays to corn has been shown to decrease the effects of aflatoxin when fed to swine. These compounds probably work by non-specific binding to the mycotoxin and reducing the rate of passage through the gut. Although not specifically approved for the purpose, various products that have this ability are approved as binding or anti-caking agents.

The diseases caused by the most relevant mycotoxins are presented in Table 38.2. Aflatoxins may cause vaccines to fail, increase the birds' susceptibility to disease, and result in suppression of natural immunity to the infection (Elissalde et al., 1989). The animals then become susceptible to infection by bacteria such as *Salmonella* and to various viruses and other infectious agents commonly found around the farm, feedlot or poultry house. Normal healthy animals would ward off such agents.

Zearalenone and zearalenol are produced almost exclusively by *Fusarium* species that contribute to the ear and stalk rot that occurs in the ears of corn and on the heads of cereal grains. When consumed by swine at more than 0.1 to 5 mg toxin per kg body weight, these compounds cause the estrogenic syndrome, which is characterized in females by a swollen and edematous vulva with enlarged mammary glands and in young males by a shrinking of the testes. Young gilts may show uterine prolapse. The financial loss to farmers comes about primarily through poor reproductive performance.

Feeds that contain 1 mg of deoxynivalenol per kg may result in significant reductions in feed consumption and weight gain by swine (Bergsj. et al., 1992). Vomiting is rather uncommon in field cases because usually pigs will not eat enough of the contaminated feed. Clinical signs and lesions in affected swine included feed refusal, a few instances of vomiting, lack of weight gain, poor feed efficiency, failure of mature sows to return to oestrus, reduced efficiency, high mortality of nursing pigs, intestinal tract inflammation, and acute diarrhoea in young pigs. Dairy cattle and poultry are relatively insensitive to the dietary concentrations of deoxynivalenol likely to be found in feeds. Apparently all

domestic animals are susceptible to injury by dietary intake of trichothecenes such as T-2, HT-2, and diacetoxyscirpenol in the region of a few mg/kg. In poultry, feed contaminated with 1 to 3.5 mg/kg of T-2 and 0.7 mg/kg of HT-2 (a closely related toxicant) may produce lesions at the edges of the beaks, abnormal feathering in chicks, a sudden and drastic drop in egg production, eggs with thin shells, reduced body weight gains, and mortality. The same feed given to turkeys results in reduced growth, beak lesions, and less immunity to infection. T-2 and DAS in cattle feed results in unthriftiness, decreased feed consumption, slow growth, reduced milk production, and sterility. An outbreak of hemorrhagic bowel syndrome and death of some animals can occur in herds of cattle and swine. In swine, infertility with some lesions in the uteri and ovaries result from consumption of feed contaminated with 1 to 2 mg/kg of T-2 toxin. As with most other mycotoxins, the only control is to avoid use of contaminated feeds. Equine leucoencephalomalacia occasionally occurs in horses, mules, or donkeys foraging corn left standing in the field after harvest or when fed grain or screenings heavily infected with *F. moniliforme*. The toxins, fumonisin B1 and B2, are produced only by certain strains of *F. moniliforme*. This toxicant is also carcinogenic in laboratory tests.

Ochratoxin A, produced primarily by members of the *Aspergillus ochraceus* group and a number of species of *Penicillium*, especially *P. viridicatum* has been found in some samples of feed grains. Frequently, citrinin is produced simultaneously by these same fungi. In the field, however, injury from ochratoxin poisoning has occurred chiefly (or only) in poultry and swine. Listlessness, huddling, diarrhea, tremors, and other neural abnormalities are sometimes encountered in broiler flocks. Ochratoxin damage to the kidneys of swine is characteristic enough to be called "porcine nephropathy," which is recognizable in commercial slaughtering.

4.3 Intoxications by other components. In addition to mycotoxins and naturally occurring toxic constituents in plants, there are toxic and deleterious substances that are extraneous contaminants of industrial origin. These can be increased to abnormal levels in animal feed through mishandling or other factors. The most significant hazards to human health are those chemicals that accumulate in animal tissues, are excreted in milk or become incorporated in eggs. Examples of substances that attract international attention are the polychlorinated biphenyls (PCBs), dioxins and furans and certain pesticides like DDT (Dichloro-Difenyl-Trichloorethane). Fish and fish by-products that are used to make fish meal and oil, and are ingredients in feed, may be sources of contamination in the food chain. For an overview in 1998/1999 a survey was conducted by the Canadian Food Inspection Agency. For an overview visit the internet page www.inspection.gc.ca/english/anim/feebet/dioxe.shtml. The purpose of the survey was to determine the levels of dioxins and furans, PCBs and DDT in fish meals, fish feeds and fish oils. The results indicated that dioxin-furan and PCB levels in fish feed and fish meal would not be expected to result in fish products with dioxin-furan or PCB levels above the Canadian guidelines for these chemical contaminants. These are a maximum level of 20 ng TEQ for dioxin and furan and 2.0 mg for PCBs per kg product. The same applies to fish oil. Also the levels of DDT were far below the maximum Canadian limit of 5.0 mg per kg product.

Other contaminants include heavy metals such as lead, mercury and cadmium. In a Dutch survey carried out in 1998 (http://www.agralin.nl/kap/kap98/kap98_4.html) median levels were less than 1 % of the cadmium limit of 1 mg per kg product for soybean husks, soybean forage, maize gluten feed, palm kernel husks and citrus pulp. Cadmium levels in mineral mixes also complied with the limit set for cadmium.

Another group of contaminants includes veterinary drugs that are administered via animal feeds. If the concentration used is high or withdrawal periods are not properly observed, foods of animal origin may contain residues that exceed established maximum residue limits (MRLs), such as those established by the CAC, and there may be a potential risk to human health. The problem can be avoided by applying good veterinary practices (GVP).

4.4 Zoonotic feedborne diseases. Epidemiological analysis of foodborne human diseases in The Netherlands for example, shows that the majority of cases of food-related gastro-enteritis involve

bacterial infections contracted from foods of animal origin. For the Dutch situation, the working group of experts of the Health Council of the Netherlands estimated that up to 75% of foodborne diseases are transmitted through products of animal origin (Health Council, 2000). The figure is based on the assumptions of experts. Human disease causing viruses are not believed to be of animal origin. As far as parasites are concerned, a small but unknown proportion may be caused by these organisms, although this may differ from country to country.

The most relevant organisms involved in foodborne diseases transmitted via products of animal origin include *Campylobacter* spp., *Salmonella enterica*, *Clostridium perfringens*, *Staphylococcus aureus*, *Bacillus cereus*, pathogenic *Escherichia coli* and *Yersinia enterocolitica*. All these organisms may originate from animal feed. The most two important organisms are described shortly below.

***Campylobacter* spp.** The species of greatest concern is *Campylobacter jejuni* which is a Gram-negative, slender, curved, motile rod. It is a micro-aerophilic organism, which means that it has a requirement for reduced levels of oxygen. It is relatively fragile, and sensitive to environmental stresses (e.g., 21% oxygen, drying, heating, disinfectants, acid conditions). The organism is especially sensitive to drying. Its main habitat is the intestinal tract of warm-blooded animals, especially the caecum of chickens, where $> 10^6$ / g *C. jejuni* may be present. The organism does not multiply at temperatures < 30 °C.

Surveys have shown that *C. jejuni* is the leading cause of human bacterial diarrhoeal illness, not only in the Netherlands but in many other countries, including the United States.

There are several routes by which animals can become colonised by *C. jejuni*. Giving animals untreated water is a common source. Contamination may also occur by feeding green crops since these may become contaminated by birds that shed *C. jejuni* in their faeces. *Campylobacter* has been isolated very rarely from animal feeds like silage, cereals, or dry compound feeds. This is in accordance with the above indicated characteristics of the organism to dry conditions and susceptibility to environmental stress.

***Salmonella enterica*.** The organism is a rod-shaped, motile bacterium, non-spore forming and Gram-negative. *Salmonella* is primarily present in the intestinal tract of animals. Where feed is the source, epidemiological data show clearly that the route of transmission is animal feed _ product of animal origin _ human infection (Crump et al, 2002). The organism can be present in raw meat and poultry, raw seafood, eggs, milk, dairy products, frog legs, yeast, coconut, sauces, salad dressing, cake mixes, cream-filled desserts, cream toppings, dried gelatine, peanut butter, cocoa, and chocolate.

Salmonella is a ubiquitous organism. It survives for long periods in natural environments and is resistant to e.g. dry conditions. As a consequence it can be present in animal feed and raw materials used in animal feed.

In an epidemiological study carried out in Denmark, an assessment was made of factors that contribute to the risk of *Salmonella* contamination in poultry flocks (Angen et al., 1996). The study revealed that the following factors (in order of importance) contributed significantly to the contamination of poultry with *Salmonella*:

- Origin of the day-old chicks.
- Company delivering the feed.
- Number of flocks present on the farm.
- Season of the year (more positive flocks in autumn).
- Presence of *Salmonella* in previous flock(s).

Although several routes of transmission can be identified, animal feed is still thought to be a major source of *Salmonella* infection in poultry flocks. Therefore, efforts continue to be made to reduce the incidence of *Salmonella* in feeds, given to poultry and other animals. There is no lack of knowledge or literature on processing methods and control measures for this purpose (Beumer, 1996; Beumer and Van der Poel, 1997). In the United Kingdom, there has been some success in reducing the *Salmonella* content of feed and data for the period 1989 -1995 show a steady decrease in *Salmonella* contamination (Report of CVL, New Haw, Addlestone, Surrey, KT15 3NB. Tel. + 44 1932 341111 Fax + 44 1932 349983)

5. Animal welfare and feed. Feed plays an important part in animal welfare, as mentioned previously. Also the composition of feed is important. Feeding of pregnant sows on high-fibre diets appeared to reduce feeding motivation and thus improved the welfare of animals (Ramonet, et al., 1999). Supplying calves with straw-kecal pellets is considered beneficial for the physiological aspects of welfare in veal calves (Morisse et al., 1999), and judicious use of disease-preventing additives also contributes to animal welfare. However, unrestricted use of antimicrobials to compensate for poor husbandry is detrimental. A major obstacle to the judicious use of feed additives, is the lack of unbiased information on their efficacy and safety for farm animals in the scientific literature (Kan, et al., 1998). Welfare requirements alone make considerable demands on feed safety and feeds should not contain any infectious agents (bacteria, parasites and viruses) that could cause illness and discomfort in the animals. Toxic components such as naturally occurring plant toxins, anti-nutritional factors and mycotoxins should be controlled at levels that would avoid harm to the animals. Balanced formulations, well controlled production technologies and quality and safety management in the feed industry should ensure that all nutritional requirements can be satisfied. However, feeds and feeding systems may also contribute to keeping animals under conditions that allow more natural behavior and thereby reduce stress associated with high-performance animal production. Drinking systems also have clear effects on animal health and welfare. For example, it was demonstrated by Turner et al. (1999) that aggression at the drinkers was greater for large groups of growing pigs when drinker allocation was restricted. Laitat et al. (1999) compared a 'tube-type' feeder, by which a mixture of meal and drinking water could be given to weaned pigs, with another type of feeding system where drinking and eating were separated activities. It was observed that feeding behaviour and hence welfare were influenced by the type of feeder used, especially with large numbers of animals. The tubetype feeder resulted in a 'more relaxed' response. For lactating cows, it was observed that grooming, which is a behavioural need, increased significantly when feed cows were not locked up and had free access to feed (Bolinger et al., 1997).

6. Proactive control. The traditional approach to controlling the safety of animal feed safety is based largely on practical experience, education and training of personnel, inspection of production facilities and operations, and testing of the finished product. End product testing is usually an integral part of the overall control programme. However, leaving aside questions regarding the accuracy and reproducibility of the methods used, it has become clear that testing of feed is of limited value without a sound sampling plan. For the production of safe food a successful pro-active control programme is now in use. It is essentially based on the application of two basic systems: Good manufacturing Practise (GMP) and the Hazard Aanlysis Critical Control Point (HACCP)-concept. For the production of safe animal feed such a system needs yet to be developed.

6.1 GMP. One of the first quality assurance systems developed by the food industry was that involving the application of GMP, as a supplement to end-product testing. GMP is considered now as a prerequisite for safe food production and has been used for many years to ensure the microbiological and chemical safety and quality of food. The establishment of GMP is the outcome of long practical experience and attention to environmental conditions in the food plant, e.g. requirements for plant layout, hygienic design of equipment and control of operational procedures. GMP is now being introduced into the feed industry for the production of safe animal feed. This measure is supported by the FAO report on animal feeding and food safety (1997) which recommends that GMP is followed at all times in the production of animal feed. Thus specific control measures are given for identified hazards, which include TSEs, biological agents, veterinary drugs, agricultural chemicals and mycotoxins. However, the GMP concept is largely subjective and its benefits are only qualitative Also, it has no direct relationship to the safety status of the product. For these reasons, the concept has been extended by introducing the HACCP system, which seeks, among other things, to avoid reliance on testing of the end-product as a means of controlling food safety. As indicated previously, such testing may fail to distinguish between safe and unsafe batches of food and is both time-consuming and relatively costly.

6.2 HACCP-system. The HACCP concept is a systematic approach to the identification, assessment and control of hazards in a particular food operation. It aims to identify problems before they occur and establish measures for their control at stages in production that are critical to ensuring the safety of the food. Control is proactive, since remedial action is taken in advance of problems occurring.

The full HACCP system, as described in Alinorm 97/13, is shown in Table 2. The document also gives guidelines for practical application of the HACCP system. The system became a legal requirement for the production of all food products in the European Union (Directive 93/43).

Despite widespread usage, the present HACCP concept still has some weak points. One of them is the definition of a hazard. This is not defined as "an agent with the potential to cause an adverse health effect", as is usual in risk assessment, but as "an unacceptable contamination, growth and/or survival by micro-organisms of concern" (ICMSF, 1988), which is more restrictive and does not cover all possible hazards. Another weakness arises from the definition of a CCP. It is stated that a CCP is a location, practice or procedure where hazards can be minimised (ICMSF, 1988; IAMFES, 1991) or reduced to an acceptable level. In both cases, these are qualitative objectives and may lead to differing interpretations. It was Notermans et al. (1995) who first made a plea to use the principles of quantitative risk assessment for setting critical limits at the CCPs (process performance, product and storage criteria). It was their opinion that only when the critical limits are defined in quantitative terms can the level of control at CCPs be expressed realistically

| Principle | Activity |
|------------------------------------|--|
| 1 Conduct a hazard analysis | List all potential hazards associated with each step, conduct a hazard analysis, and consider any measures to control identified hazards |
| 2 Critical Control Points (CCPs) | Determine Critical Control Points (CCPs) |
| 3 Critical limit(s) | Establish critical limits for each CCP |
| 4 Monitoring | Establish a system of monitoring for each CCP |
| 5 Corrective actions | Establish the corrective action to be taken when monitoring indicates that a particular CCP is not under control |
| 6 Verification procedures | Establish procedures for verification to confirm that the HACCP system is working effectively |
| 7 Documentation and record keeping | Establish documentation concerning all procedures and records appropriate to these principles and their application |

Table 2. *The seven principles of the HACCP-system, (CAC, Committee on Food Hygiene, 1997)*

6.2.1 From feed safety objectives to HACCP-criteria. A critical control point in a feed production process can be defined as a location, practice or procedure where hazards can be minimised or reduced to an acceptable level. Therefore, the identification of CCP's in a feed production process is an important step in the control of such hazards. There are various means for controlling potentially hazardous bacteria and chemical agents in feed production processes. Suitable control of hazardous chemical agents can be achieved by setting appropriate criteria for raw materials. Hazardous micro-organisms can be inactivated by e.g. heating or irradiation, while acidification of feed and use of controlled storage conditions, etc. may also be of value. In some cases stabilisation of microbial levels i.e. prevention of growth, may be sufficient. Stabilisation can be achieved by adjusting the

formulation to give a low aW, pH, etc. Such measures not only stabilize bacterial populations, but can also reduce the numbers of any pathogens present. In summary, there are several options to produce safe animal feed. They comprise:

- setting requirements (criteria) for the raw materials used
- setting criteria for processing (e.g. heat treatment)
- composition of the feed material (e.g. pH, aW)
- setting storage conditions

For the relevant possibilities, criteria need to be set in advance. For example, if a dry feed product is being produced from wet raw materials and *Salmonella* could be present, a drying process should be defined that effectively kills *Salmonella*. If an acidified feed is required a pH range should be selected that does not allow multiplication of any hazardous organisms. For feed with a limited storage life, storage conditions need to be established, including temperature and maximum storage time. If there are no CCP's in the production process, they should be introduced! GMP and HACCP are now designed to meet the criteria set. The role of GMP is to ensure that hygienic equipment is used, that well trained personnel are involved in the production process, that re-contamination is avoided, etc. HACCP is the managerial tool in assuring that the chosen criteria are met. Finally, verification that GMP and HACCP work as planned needs to be introduced.

6.3 Shortcomings of the control system. In the HACCP system recalls are part of the '*corrective actions*'. These actions are necessary when monitoring indicates that a particular CCP is not under control. If a failure is only apparent after the product has been sold a recall action prevents the anticipated health problem in either humans or animals. Incidentally, GMP and HACCP do not, in them selves, prevent specific health problems and corrective actions are necessary. Such situations may occur, for example, when unknown hazards appear or in the case of rare hazardous events. The same may apply where malicious practices or carelessness have occurred.

Unknown hazards. GMP and HACCP are largely based on knowledge collected in the recent past. The system may be inadequate if new, unknown hazards occur or when a hazard re-emerged. Recent examples are the internal contamination of eggs with *S. Enteritidis* and contamination of beef burgers with *E. coli* O157. Before 1980, *E. coli* O157 was unknown as causes of foodborne diseases and after 1980 *S. Enteritidis* re-emerged as disease. A newly discovered chemical hazard is acrylamide which arises from the heating of certain food products. Once these 'new' hazards have been characterised they will be introduced into the GMP and the HACCP concept for relevant applications.

Rare hazards. There are many types of micro-organisms and even more chemical substances that can have an adverse effect on human and animal health. Hazards that occur only very rarely are not usually included in GMP and the HACCP concept. Control of these hazards is mostly carried out at national level by general measures on the part of government. Dioxin is such an example, and the government took general measures to reduce the probability that this toxic substance would spread in the environment. One of these measures was to prohibit the production of food and feed in a given area after contamination of that area had been established.

Corrupt practices. Corrupt or malicious practices are activities which are forbidden by law. A recent example was deliberate use of sugar waste which contaminated the hormone medroxyprogesteron acetate as a raw animal feed material. Malicious practices would include terrorist activity, for example, in the case of deliberately induced food poisoning.

Carelessness. Although GMP and HACCP aim to prevent human failings they sometimes fail themselves because of carelessness. Errors or failings occur when staff are stressed by pressure of work or temporary and poorly trained people or motivated personnel are employed to do the work.

It is clear that control may be lost. The GMP and HACCP concept take this into consideration by corrective action. If a problem is recognised before distribution, the product can be retained until the corrective action has been carried out. In the case of a product that has already been sold than then a recall is necessary.

For a successful recall, the product in question must be traced rapidly. Therefore an adequate system of traceability is an additional requirement in the production of safe food and feed. Since recalls may be very costly for the producer an early warning system to reduce the probability of a recall would be beneficial.

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Fermented Liquid Feed (FLF) can reduce the transfer and incidence of *Salmonella* in pigs.

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Summary. Surveillance studies have shown that feeding pigs liquid diets, and particularly fermented liquid diets reduces the incidence of *Salmonella* positive herds. Studies have shown that a concentration

of 70 mmol kg⁻¹ lactic acid is bacteriostatic to *Salmonella* and that concentrations >100 mmol kg⁻¹ are bactericidal. Uncontrolled natural fermentation results in lactic acid concentrations varying between 0 and 140 mmol kg⁻¹ so cannot be relied upon to produce bactericidal levels of lactic acid. However, if selected lactic acid bacteria are used as inoculants and the temperature of the fermentation is controlled (circa 30°C), acid conditions can be produced within 24 h that rapidly and effectively exclude enteropathogens from the diet.

FLF has beneficial effects on the gut architecture and significantly reduces coliform numbers in the lower gut. Sows fed FLF, fermented using pig-derived LAB, have reduced numbers of coliforms in their dung. Piglets suckling sows fed FLF have reduced numbers of faecal coliforms and the LAB species used for fermentation can be recovered from the piglets' faeces. The colostrum from sows fed FLF has increased immunoglobulin concentration and increased mitogenic activity for both lymphocytes and epithelial cells. This suggests that feeding sows FLF could reduce vertical transmission of enteropathogens.

Taken together, these results indicate that FLF can reduce the potential for enteropathogen transfer via the food, can beneficially influence the ecophysiology of the gut and can stimulate the pig's immune system.

Health benefits of liquid feeding systems.

In Europe, the animal feed industry has reduced the incidence of *Salmonella* in feed by stringent quality control and the use of high temperature treatments to kill any residual *Salmonella* in raw materials. Despite this, there is growing evidence that this approach has been unsuccessful in reducing the incidence of *Salmonella* in pigs on production units. The incidence of *Salmonella* is lower when pigs are fed liquid diets than when they are fed dry and particularly pelleted diets (Tielen et al., 1997; United States Animal Health Association, 1999; van der Wolf et al., 1999; von Altröck et al., 2000; van der Wolf et al., 2001; Wong et al., 2002). The incidence was particularly low on farms that fed acidified cheese whey (Tielen et al., 1997) or fermented food industry co-products (van der Wolf et al., 1999).

As Scholten et al. (1999) have pointed out the majority of food industry co-products have been fermented by lactic acid bacteria and as a result have a low pH and contain significant quantities of lactic acid. This high lactic acid concentration inhibits *Salmonella* in the feed (Geary et al., 1999; Beal et al., 2002) and hence eliminates it at the start of the food chain. Consequently, the inclusion of fermented co-products in liquid diets for pigs makes a significant contribution to food safety. Producers who do not have access to liquid co-products can gain similar benefits by using traditional dry diets if these are fed in liquid form and fermented with lactic acid bacteria (LAB).

Virtually any combination of feed ingredients will ferment if left to steep in water. Almost all raw materials have a natural flora, which includes potentially beneficial LAB and yeasts. Many may also have an undesirable microflora, which can include coliforms, *salmonellas* and moulds. In the initial stages of fermentation there is a 'blooming' of coliforms (Hansen and Mortenson, 1989; Russell et al., 1996; Geary, 1997) followed by a proliferation of LAB, which normally dominate natural fermentations. However, at low operating temperatures and particularly in some feed ingredients (e.g. by-products from brewing and ethanol production) yeasts will dominate. As they can tolerate low pH yeasts will nearly always become a dominant feature of natural fermentations over time. Yeast fermentation is not desirable as starch is turned into alcohol and carbon dioxide reducing the energy value of the feed. In addition, fermentation by inappropriate yeasts can produce 'off' flavours and taints that can make the food unpalatable.

Fermentation can reduce the incidence of enteropathogens in liquid feed.

As noted earlier, the animal feed industry makes strenuous efforts to reduce the incidence of enteropathogens (particularly *Salmonella* spp.) from dry diets. However, no matter how effective this

process is, there remains the possibility that the feed can become re-contaminated between leaving the mill and being eaten by the pig. An advantage of properly fermented liquid feed is that the acid content of the feed significantly reduces the risk of re-contamination.

Salm. typhimurium DT104:30 was rapidly excluded when it was introduced into feed that has been fermented for >48 h with *Pd. Pentosaceus* (Beal et al., 2002). However, the death rate was very temperature dependent. *Salm. typhimurium* DT104:30 died four to five times faster in feed maintained at 30 °C (D_{value} 34-45 min) compared with feed maintained at 20 °C (D_{value} 137-250 min). It appears that this effect is due to the lactic acid concentration of the fermented feed rather than the presence of any bacteriocins produced by LAB (Geary et al., 1999; van Winsen et al., 2000).

An important practical consideration is the rate at which potential enteropathogens are excluded at the start of the fermentation process. As noted earlier, fermentations are characterised by an initial coliform bloom and this is only reversed when lactic acid levels rise. Studies in our laboratory have shown that when *Salm. typhimurium* DT104:30 and *Pd. pentosaceus* are co-inoculated into liquid feed the *Pd. pentosaceus* rapidly dominate the fermentation and reduce *Salm. typhimurium* to undetectable levels (Beal et al., 2002). However, this effect is also temperature dependent. The decimal reduction time (D_{value}) of *Salmonella* was significantly better at 30 °C (D_{value} 34-45 min) than at 20 °C (D_{value} 137-250 min). It was found that a lactic acid concentration of 70 mmol kg⁻¹ was bacteriostatic, but higher levels (>100 mmol kg⁻¹) were needed in order to be bactericidal.

Studies in our laboratory have demonstrated that FLF is also effective in excluding a wide range of potentially pathogenic coliform bacteria from FLF (see paper by Beal et al. at this conference).

Unfortunately, natural fermentations have produced unpredictable results on commercial units. Recent studies (Beal, Niven and Brooks in press) have provided an explanation for this. Samples of wheat and barley were obtained, at harvest, from across the UK. These samples were allowed to ferment and their acid production was assessed. There was a great deal of variation in the amount of lactic acid produced by the samples. After fermentation for 24 h at 30 °C lactic acid concentration ranged from 0.14-134.9 mmol kg⁻¹ (mean 59.6±40.0). Only 3% (9 of 300) of fermentations conducted produced more than 75 mmol kg⁻¹ after 24 h fermentation. Thus natural fermentations, which rely on the indigenous flora present on grains, cannot be relied upon to produce bacteriocidal levels of lactic acid.

More predictable fermentation can be achieved by the inoculating liquid feed with LAB that produce lactic acid rapidly and have a high terminal lactic acid concentration. Beal, Niven and Brooks (unpublished data) have identified a number of LAB species are capable of producing 180-230 mmol kg⁻¹ lactic acid in 24 hours with <30 mmol kg⁻¹ acetic acid (a low level of acetic acid is needed to maintain palatability). Regrettably, these organisms cannot be used as inoculants in the EU because of an anomaly in the current legislation (Brooks et al., 2003)

Fermented feed or fermented feed components?

A number of studies have been undertaken using continuous fermentation of complete diets (i.e. a proportion of the feed is retained each day to act as an inoculum for the next day's feed) (Geary et al., 1996; Russell et al., 1996; Jensen and Mikkelsen, 1998; Geary et al., 1999; Pedersen, 2001; Pedersen et al., 2002a; Scholten et al., 2002). However, although some of these gave good results it is difficult to obtain a reliable and consistent fermentation. Concern has also been expressed that fermenting complete diets could reduce nutrient availability. Specifically, synthetic amino acids added to the diet have been degraded in the fermentation process (Pedersen, 2001; Pedersen et al., 2002b; Pedersen et al., 2002c). If the diet contains no synthetic amino acids fermentation does not appear to have any adverse effect on lysine levels (Pedersen, 2001). Studies in our laboratory indicate that LAB used as inoculants do not degrade lysine, and that the loss of synthetic lysine may result during

the coliform bloom when there could be an acid induced activation of the adaptive pathway involving lysine decarboxylase possessed by *Salmonella* and *E.coli* (Meng and Bennett, 1992; Park et al., 1996). This is supported by our observation that uncontrolled fermentations result in a significant increase in the production of biogenic amines (Niven unpublished data).

Fermenting only the cereal fraction has significant practical advantages compared with fermenting complete diets. However, if only the cereal component is fermented, a higher concentration of acid must be generated (in order to compensate for dilution and buffering effects when the complete diets is produced) and this can only be achieved using selected LAB as inoculants to stabilise the process.

Effects of FLF on the gastrointestinal tract.

Young pigs have an insufficiency of stomach acid, which is the first line of defence against bacterial invasion (Smith and Jones, 1963; Cranwell et al., 1976). Feeding FLF reduces gastric pH and the number of coliforms in the stomach (Mikkelsen and Jensen, 1997; Moran, 2001; van Winsen et al., 2001; Scholten et al., 2002; Canibe and Jensen, 2003). This is due not only to the concentration of acid but also to high concentration in an undissociated form (Russell and Diez-Gonzalez, 1998; van Winsen et al., 2001). Similarly, chickens fed FLF had reduced susceptibility to infection with *Salmonella* (Heres et al., 2003a) and this was attributed to the barrier function provided by increased acidity in the gizzard and proventriculus (Heres et al., 2003b).

Post weaning anorexia can have a significant effect on the villous architecture of the pig and this can be reduced by feeding liquid diets - see review by Brooks et al. (2001). Furthermore there has been evidence in some studies that feeding FLF has additional benefits in maintaining gut architecture (Scholten et al., 1999; Moran, 2001; Scholten et al., 2002). This may result from a combination of factors including, improved intake of the liquid diet, reduced viscosity of feed and digesta, alterations to the nutrient supply for the lower gut microbiota and the probiotic or immunostimulatory properties of the LAB present in the FLF

It has been demonstrated that increasing digesta viscosity predisposes pigs to coliform proliferation (Hopwood et al., 2002). Liquid feed and particularly FLF, has reduced viscosity (Niven and Brooks unpublished data) and reduces the dry matter content of the digesta (Canibe and Jensen, 2003). These physico-chemical differences may contribute to the observed changes in the eco-physiology of the pig's GIT.

Generally, feeding FLF does not produce any significant increase in the number of lactic acid bacteria present in the GIT (after the stomach) but it does dramatically reduce the number of coliforms in the lower small intestine, caecum and colon (Jensen and Mikkelsen, 1998; Hansen et al., 2000; Moran, 2001; van Winsen et al., 2002; Canibe and Jensen, 2003). The ratio of lactic acid bacteria to coliforms in the lower gut of pigs weaned onto freshly prepared (non-fermented) liquid diets was very similar to that of pigs that continued to suckle the sow. However, when the pigs were weaned onto dry diets there was a significant shift in the ratio towards the coliform bacteria. Conversely, when pigs were weaned onto FLF the number of coliforms was reduced and the ratio shifted in favour of the lactic acid bacteria (Moran, 2001).

Recently, van Winsen et al. (2002) conducted a longitudinal study to measure the effect of fermented feed, in particular of its components lactic acid and *Lactobacillus plantarum*, on gastrointestinal bacterial ecology. Their results showed that fermented feed reduced the Enterobacteriaceae population in the faeces of pigs, which is supported by studies in our laboratory.

At the University of Plymouth we have been using this effect with a different objective. The newborn pig usually has a sterile gut at birth and acquires its characteristic flora through contact with its mother and its surroundings. The most significant contributor of bacteria to the piglet's surroundings

is the sow. Therefore, we reasoned that if the gut microflora of the sow could be manipulated this would impact on the development of the piglet's gut microflora. To this end sows have been fed diets fermented with aggregating LAB derived from healthy sows and compared them with sows fed dry diets or non-fermented liquid diets prepared immediately before feeding (Demecková et al., 2002). The treatments had no effect on the number of LAB in sows' faeces, but feeding FLF significantly reduced the number of coliforms shed. The faeces of piglets suckled by sows fed FLF contained significantly more lactic acid bacteria (7.7 vs. $7.3 \log_{10}$ CFU g^{-1}) and significantly less coliforms (7.5 vs. $8.1 \log_{10}$ CFU g^{-1}) than the faeces of piglets suckling sows fed dry feed. The colostrum of sows fed FLF had increased immunoglobulin activity and increased the mitogenic activity of lymphocytes and enterocytes (see paper by Demecková et al. in this publication).

A detailed discussion of the reported immunostimulatory effects of LAB is beyond the scope of this paper and raises important issues about when LAB inoculants become probiotics. This inevitably raises more legislative issues. However, it is clear that the immunostimulatory effects depend on the both the organism used and the dose (Donnet-Hughes et al., 1999; Gill and Rutherford, 2001), and generally require continued ingestion. Dose levels required to produce an immunostimulatory effect appear to be of the order 10^9 CFU. This level is consistent with daily dose of *Lab. plantarum* provided by FLF.

Conclusions

Liquid feeding appears intrinsically to reduce the incidence of *Salmonella* in pigs. The inclusion of acidic components in diets and the controlled fermentation of liquid feed can provide a simple mechanism whereby the bio-safety of feed can be increased. The ability of fermented feed to exclude pathogens such as *Salmonella* could make an important contribution to food safety. Furthermore, the immunostimulatory effects of lactic acid bacteria could be harnessed to improve gut health following the reduction in the use of antibiotic growth promoters. This capability will increase in importance as legislators press the pig industry to remove antibiotic growth promoters from their diets. However, it is the legislators who will also determine whether the potential of LAB and fermented feed will be realised. Current EU legislation is preventing the development of this technology and unless this problem is addressed in a rational and constructive way an extremely useful technology may fail to be implemented on commercial units.

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Emerging Antimicrobial Resistance in Foodborne Pathogens

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Summary: Foodborne microbial illnesses are an important public health issue worldwide. Although these illnesses are usually a mild to moderate self-limiting gastroenteritis, invasive diseases and complications may occur. Many foodborne bacteria (pathogenic and commensal varieties) colonize the gastrointestinal tracts of a wide range of wild and domestic animals, especially animals raised for human consumption. Food contamination with these pathogens can occur at multiple steps along the food chain, including production, processing, distribution, and preparation. An additional concern is the growing incidence of antimicrobial-resistant foodborne pathogens. This paper will focus on antimicrobial resistance among three of the most relevant foodborne bacterial pathogens, *Salmonella*, *Campylobacter*, and *E. coli*.

Keywords: *Salmonella*; *Campylobacter*; *E. coli*; Multiple antimicrobial resistance; food animals

Introduction: The fact that microbial ecosystems are interconnected must be underscored. Bacteria present today in a pig's intestine may a week later be in packaged pork products, and two months thereafter, in a community reservoir. Antimicrobial resistance in foodborne bacteria, therefore, should not necessarily be considered distinct from that in isolates from humans, food animals, or other niches. Yet because food consumption is an important pathway for bacteria to enter humans, the presence of antimicrobial-resistant bacteria in foods warrants particular attention.

Antimicrobial-resistant bacteria have been recovered from a wide variety of foods, and several thorough reviews have been written on the broad subject of antimicrobial-resistant bacteria in the food production

continuum. The specific purpose of this paper is to summarize antimicrobial resistance among three of the most relevant foodborne bacterial pathogens, *Salmonella*, *Campylobacter*, and *E. coli*.

Salmonella

Antimicrobial resistance among *Salmonella* isolates is increasing on a global scale. A recent 7-year study in Spain revealed that ampicillin resistance in *Salmonella* species has increased from 8% to 44%, tetracycline resistance from 1% to 42%, chloramphenicol resistance from 1.7% to 26%, and nalidixic acid resistance from 0.1% to 11% (Prats et al. 2000). Similarly, in Great Britain, the reported rates of antimicrobial resistance for *S. Typhimurium* more than doubled between 1981 and 1989 (Threlfall et al. 1993). In the United States, resistance to tetracycline has increased from 9% in 1980 to 24% in 1990 and ampicillin resistance increased from 10 to 14% (Lee et al. 1994). Fluoroquinolones and expanded-spectrum cephalosporins, the primary antimicrobials for treating human infections caused by multidrug-resistant strains (Cherubin & Eng 1991; Fey et al. 2000), are also showing decreased activity against *Salmonella* species (Fey et al. 2000; Winokur et al. 2000).

An important factor in this increase in resistance has been the epidemic spread through food animals and humans over the past 10 years of multidrug resistant *S. Typhimurium* DT104 (Glynn et al. 1998; Threlfall et al. 1993). DT104 is characterized by a resistance to five agents: ampicillin, chloramphenicol/florfenicol, streptomycin, sulfonamides, and tetracycline (ACSSuT). This strain type was first isolated from sea gulls, then cattle and later in humans in England. Since then, it has been isolated from multiple animal species including poultry, cattle, pigs, sheep, and non-domestic birds (Besser et al. 2000). Some strains in Great Britain have acquired additional resistance to trimethoprim, aminoglycosides as well as decreased susceptibility to fluoroquinolones (Threlfall et al. 1996). The majority of resistant DT104 isolates have a unique multidrug resistance chromosomal gene cluster encoding the complete spectrum of the ACSSuT phenotype (Threlfall et al. 1996). This gene cluster typically consists of a chromosomal locus 12.5 kb in size with flanking integrons (Briggs & Fratamico 1999). The first integron possess the *aadA2* gene, conferring resistance to streptomycin and spectinomycin; the second contains the β -lactamase gene *blaPSE-1* encoding resistance to ampicillin. A gene encoding sulfonamide resistance (*sul-1*) is present within conserved sequences of both integrons. Flanked by these two integron structures are the *flo* efflux gene, conferring cross-resistance to chloramphenicol and florfenicol, and the tetracycline resistance genes *tetR* and *tetA* (1999; Briggs & Fratamico 1999). The DT104-like resistance gene cluster has also been described in poultry strains of *S. Agona*, suggesting that it is transmittable between serotypes. It has been shown experimentally that the DT104 MDR cluster can be efficiently transduced by P22-like phages. In addition, upstream of the first integron in the MDR locus is a gene encoding a putative resolvase enzyme, which demonstrates greater than 50% identity with the Tn3 resolvase family. This suggests that the MDR antibiotic resistance gene cluster could be part of a much larger transposon.

The presence of resistant *Salmonella* in retail meats has been assessed in numerous studies. In a pilot survey by White et al., (White et al. 2001) two hundred ground meat samples (51 chicken, 50 beef, 50 turkey, and 49 pork) were purchased from retail stores representing three different supermarket chains in the greater Washington DC area between June and September of 1998. Products were fresh, prepackaged meats coming from four poultry, and one pork processing plants, and store-ground and packaged beef. *Salmonellae* were recovered from 41 of 200 (21%) ground meat samples. *Salmonella* was isolated more frequently from poultry (33% of chicken and 24% of turkey samples) than red meats (18% of pork and 6% of beef samples). All *Salmonella* were susceptible to amikacin, apramycin, ciprofloxacin and nalidixic acid. Eighty-four percent (38/45) of isolates displayed resistance to at least one antibiotic. The most common resistance observed was to tetracycline (80%), streptomycin (73%), sulfamethoxazole (69%), and to a lesser extent ampicillin (27%). In addition, 16% displayed resistance to amoxicillin/clavulanic acid, cephalothin, ceftiofur, and ceftriaxone. Ceftiofur- and ceftriaxone-resistant *Salmonella* were isolated from ground turkey, chicken, and beef.

Antimicrobial resistant *Salmonella* have also been reported from imported foods. Zhao et al. reported the antimicrobial susceptibilities of 187 *Salmonella* isolates, representing 80 serotypes, recovered from imported foods by FDA field laboratories in 2000 (Zhao et al. 2003). Fifteen (8%) were resistant to at least one antimicrobial, and five (2.7%) were resistant to three or more antimicrobials. Nine isolates exhibited resistance to tetracycline. Four isolates also demonstrated resistance to nalidixic acid - all were isolated from imported catfish or tilapia from Taiwan or Thailand. All four nalidixic acid resistant *Salmonella* isolates possessed amino acid substitutions at the Ser83 or Asp87 position in DNA gyrase. One S. Derby isolated from frozen anchovies imported from Cambodia was resistant to 6 antimicrobials including ampicillin, amoxicillin/clavulanic acid, chloramphenicol, trimethoprim/ sulfamethoxazole, sulfamethoxazole, and tetracycline.

Studies conducted outside the U.S. have also examined foods for the presence of antimicrobial resistant *salmonellae*. One study from Spain, determined the extent of antimicrobial resistance among one hundred and twelve *Salmonella* isolates recovered from 691 samples of frozen and fresh chicken meat (Hernandez et al. 2002). Almost half of the isolates tested (46%) were susceptible to all antimicrobials tested. However, resistance was commonly seen to chloramphenicol (45%), ampicillin (35%), and tetracycline (34%). Resistance to multiple antimicrobials was observed in 44% of isolates, whereas single resistance was seen in 11%. S. Typhimurium isolates tended to be more resistant than other serotypes tested. Another study from Europe authored by Mammina et al., investigated the distribution of serotypes and patterns of drug resistance of 206 strains of *Salmonella* isolated in southern Italy. *Salmonella* were obtained between 1998-2000 from 172 samples of raw foods of animal origin, 22 fecal samples from food animals and 12 animal feed samples (Mammina et al. 2002). *Salmonellae* resistant to three or more antimicrobials were considered multi-resistant. Among non-Typhimurium isolates tested, 46 of 122 (38%) strains were categorized as multi-drug resistant. S. Typhimurium was the predominant serotype recovered with 35 of 67 (52%) isolates displaying multidrug resistance. The characteristic DT104 antimicrobial resistance phenotype of ACSSuT was identified in 17 of these isolates.

Recently, there has been a national emergence of strains of S. Newport in the U.S. known as Newport-MDRampC, which are resistant to at least nine antimicrobials, including extended-spectrum cephalosporins. In the U.S., the prevalence of Newport-MDRampC among S. Newport isolates from humans increased from 0% during 1996-1997 to 26% in 2001. At least 26 states have isolated these strains either from humans, cattle, or ground beef. Like DT104, these strains were resistant to ampicillin, chloramphenicol, streptomycin, sulfamethoxazole, and tetracycline. In addition, Newport-MDRampC isolates were resistant to amoxicillin/clavulanic acid, cephalothin, cefoxitin, and ceftiofur, and exhibited decreased susceptibility to ceftriaxone (MIC $\geq 16\mu\text{g/ml}$). In 2001, Newport-MDRampC strains were responsible for 2.6% of the more than 1 million estimated *Salmonella* infections in 2001 (Anonymous 2002b).

Campylobacter

Campylobacter has been recovered from the intestinal tracts of both wild and domestic animals. In food-producing animals such as cattle, poultry and swine, fecal *C. jejuni*/*C. coli* can be regarded as a commensal organism. As a result of fecal contact during processing, enteric bacteria frequently contaminate foods derived from animals. This is the case with *Campylobacter* (*C. jejuni*), which are present in high numbers in the poultry intestine. Consequently, *Campylobacter* can be readily cultured from most retail chicken carcasses.

In a Minnesota survey, 91 chicken meat products were purchased in the Minneapolis-St. Paul area from September to November, 1997 (Smith et al. 1999). These products came from 15 poultry-processing plants in nine states. All chicken meat products were cultured for *Campylobacter*, which were tested for susceptibility to nalidixic acid and ciprofloxacin. *Campylobacter* was isolated from 88% of the meat samples tested: *C. jejuni* was isolated from 74% of chicken samples and *C. coli* was recovered from 21% of samples tested. Ciprofloxacin resistant *Campylobacter* (in this study; MIC > 32 :g/ml) were recovered from 20% of

tested chicken samples (n=18). Using molecular subtyping methods (fla-RFLP), they further identified six of seven subtypes among *Campylobacter* isolates from both retail chicken products and quinolone-resistant *C. jejuni* isolates from humans. This data demonstrated that domestic chicken obtained from retail markets had high rates of contamination with ciprofloxacin-resistant *C. jejuni*. Furthermore, the authors identified an association between molecular subtypes of quinolone-resistant *C. jejuni* strains that were acquired domestically in humans and those found in retail chicken products.

Zhao et al. (Zhao et al. 2001a) examined 719 retail raw meat samples (chicken, turkey, pork, and beef) obtained from 59 stores of four supermarket chains during 107 sampling visits in the Greater Washington D.C. area, from June 1999 to July 2000. *Campylobacter* was detected in 71% of 184 chicken samples, and a large percentage of the stores visited (91%) had *Campylobacter*-contaminated chicken. Approximately 14% of the 172 turkey samples were culture-positive, whereas few pork (1.7%) and beef (0.5%) samples were positive. Approximately one-half (53.6%) of the isolates were identified as *C. jejuni*, 41.3% were identified as *C. coli*, and 5.1% were identified as other species. *C. coli* was more often recovered from retail turkey samples than *C. jejuni*. The most common resistance observed among the *Campylobacter* poultry isolates was to tetracycline (82%), followed by erythromycin (54%), nalidixic acid (41%), and ciprofloxacin (35%) (Ge et al. 2003). *C. coli* isolates displayed higher resistance rates to ciprofloxacin and erythromycin than *C. jejuni*. Turkey isolates, regardless of species, showed elevated resistance rates to ciprofloxacin and erythromycin than *Campylobacter* isolates from retail chickens. Eighty-seven percent of samples contained *Campylobacter* resistant to at least 2 antimicrobial agents, and 22% to at least 5 antimicrobials. Co-resistance to ciprofloxacin and erythromycin was found in *Campylobacter* from 26% of the meat samples. Multi-drug resistance, including co-resistance to fluoroquinolones and erythromycin, has been reported previously in *Campylobacter* isolated from retail meat product (Saenz et al., 2000) and from humans (Engberg et al., 2001). The finding of co-resistance to fluoroquinolones and erythromycin in *Campylobacter* is undesirable, as these two antimicrobials are generally advocated as first-line drugs for treatment of human campylobacteriosis.

In 2002, The National Antimicrobial Resistance Monitoring System (NARMS) expanded into surveillance of retail meats to determine the prevalence of antimicrobial resistance among *Salmonella*, *Campylobacter*, *E. coli* and Enterococcus. As of January, 2003, 9 FoodNet participating sites are involved (California, Colorado, Connecticut, Georgia, Minnesota, New York, Tennessee, Maryland, and Oregon). Each FoodNet site purchases a total of 40 food samples per month, consisting of 10 samples each of chicken breast, ground turkey, ground beef, and pork chops. Preliminary data show that 58% of 356 chicken breasts, 8% of 372 ground turkey, 3% of 373 ground beef samples, and 2% of 343 pork chop samples cultured positive for *Campylobacter* (unpublished data). This surveillance is ongoing and being expanded to include additional state labs.

Escherichia coli

Antimicrobial-resistant *E. coli* have been recovered from a variety of foods, including minced meat, vegetables, cakes and confectionary, custards and desserts, and milk and milk products. A number of studies outside the U.S. from the early 1980s looked for the presence in foods of antimicrobial resistant Gram-negative bacteria, including *E. coli*. One of the first studies was described by Persson et al. (Persson et al. 1980). *E. coli* was the predominant bacterium identified and was most often isolated from raw meat and egg products. All isolates were considered susceptible to streptomycin, neomycin, and trimethoprim-sulfamethoxazole. Fifty-four percent (166/308) of bacteria tested, however, were resistant to at least one of the 9 antibiotics tested, with 40% displaying multi-resistance. Resistance to nitrofurantoin and sulphaisodimidine was most frequently observed (30% and 25% respectively). The lowest numbers of resistant strains identified were recovered from custards and desserts and from raw meat products (42% and 46% respectively) and the highest from ice-cream (89%). Multi-resistant strains were recorded most frequently from pasteurized milk products, custards and desserts.

Bensink et al. examined Australian beef and pig carcasses, meat products, and frozen chickens for the presence of antibiotic resistant coliforms (Bensink et al. 1981). *Escherichia coli* was isolated from 18 of 50 beef carcasses, and only resistance to tetracycline was detected. The situation was different with *E. coli* recovered from pig carcasses, meat products, and chickens, where numerous resistance phenotypes were observed, including multidrug resistant strains. A later study by the same researchers investigated antibiotic resistance in coliforms isolated from poultry carcasses immediately after slaughter and at retail (Bensink & Botham 1983). Approximately 85% of the total of 13,858 isolates examined were found to be resistant to at least one antibiotic. Highly significant differences were found in the levels of antibiotic resistance from the 2 sources; ampicillin, chloramphenicol, and sulphonamide resistance was found more frequently in isolates from poultry at retail, while resistance to streptomycin and neomycin occurred more frequently in isolates from poultry at slaughter.

Several more recent studies have investigated specifically the prevalence of antimicrobial resistant *E. coli* in retail foods. Meng et al. determined antimicrobial susceptibilities of 118 *E. coli* O157:H7 and 7 O157:NM isolates from animals, foods, and humans in the U.S. (Meng et al. 1998). Among the 125 isolates tested, 30 (24%) were resistant to at least one antibiotic and 24 (19%) were resistant to three or more antibiotics. Cattle strains were more often resistant than other isolates tested (34%). The seven resistant food *E. coli* isolates were all recovered from ground beef. Two *E. coli* O157:NM isolates from cattle were resistant to six antibiotics: ampicillin, kanamycin, sulfisoxazole, streptomycin, tetracycline, and ticarcillin. Streptomycin was the most common antibiotic to which *E. coli* O157:H7 and O157:NM were resistant (29/30 isolates), followed by tetracycline (26 isolates). Overall, the most frequent resistance phenotype observed was to streptomycin-sulfisoxazole-tetracycline, which accounted for over 70% of the resistant strains.

Sáenz et al. investigated the prevalence of antimicrobial resistant *E. coli* from animals, foods and humans in La Rioja, Spain (Saenz et al. 2001). Food products of animal origin sampled for the presence of antimicrobial resistant *E. coli* included hamburger, sausage, chicken, and turkey. Using disk diffusion, they reported that among 47 *E. coli* isolates recovered from foods, 53% were resistant to nalidixic acid, 47% to ampicillin, and 40% to kanamycin. Resistance was also observed, but to a lesser extent, to gentamicin (17%), ciprofloxacin (13%), and amoxicillin-clavulanic acid.

Schroeder et al. (2003), reported antimicrobial susceptibilities among 472 generic *Escherichia coli* isolates recovered from ground and whole retail beef, chicken, pork, and turkey obtained from greater Washington, DC, during the years 1998 and 2000. Isolates displayed resistance to tetracycline (59%), sulfamethoxazole (45%), streptomycin (44%), cephalothin (38%) and ampicillin (35%). Lower resistance rates were observed for gentamicin (12%), nalidixic acid (8%), chloramphenicol (6%), ceftiofur (4%) and ceftriaxone (1%). Sixteen percent of the isolates displayed resistance to one antimicrobial, followed by 23% to two, 23% to three, 12% to four, 7% to five, 3% to six, 2% to seven and 2% to eight. Other studies demonstrate that *E. coli* recovered from foods are not necessarily resistant to multiple antimicrobials. Zhao et al. tested 404 fresh ground beef samples obtained at retail stores from New York, San Francisco, Philadelphia, Denver, Atlanta, Houston, and Chicago for the presence of *Salmonella* and *E. coli* (Zhao et al. 2002). Among the 102 generic *E. coli* isolates obtained, only three were resistant to multiple antibiotics.

There is still debate on the public health implications of the presence of antimicrobial-resistant *E. coli* in foods. Nevertheless, the numerous reports that have documented the presence of antimicrobial resistant *E. coli* in retail foods highlights the public health value of continuing efforts to educate consumers in proper food handling and preparation practices.

Conclusions: Antimicrobial resistant bacteria from animals, both commensal and pathogenic variants, can reach the general public via exposure to contaminated food products of animal origin if they are improperly cooked or otherwise mishandled. It has been theorized that these resistant bacteria

have the potential to colonize humans and/or transfer their resistance determinants to resident constituents of the human microflora, including pathogens. Therefore, it is necessary to continue work to advance food processing technologies while emphasizing hygienic food handling at all stages of food production. There is also a continuing need for education programs aimed at improving food safety behaviors of consumers of all ages. Lastly, surveillance programs designed to detect emerging antimicrobial resistance phenotypes among foodborne pathogens in retail foods will continue to be a foundational tool for helping to ensure a safe food supply.

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Sensitivity of cultivation of *Salmonella enterica* in pooled samples of pig faeces

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Summary: We aimed investigate if the cost of bacteriological examination at herd level could be reduced by cutting down on the number of analyses without loss of sensitivity. Faeces samples sent to the lab for bacteriological examination were analysed by both the standard procedure (20 pen samples) and by mixing the same 20 samples into 10, 5 or 1 pooled sample(s), respectively. The relative sensitivity of the bacteriological analysis decreased to 94% following pooling from 20 into 10; to 92% by pooling from 20 into 5; and to 73% following pooling from 20 into 1. Percent agreement between the standard procedure and pooled samples was >90% if only *Salmonella*-negative samples, or only *Salmonella*-positive samples, were pooled. Agreement was >60% for pools of originally negative and positive samples. The suggested alternative pooling methods should be carried out in the laboratory.

Keywords: bacteriological analysis, pooling, sensitivity, pig faeces

Introduction: In the Danish *Salmonella* surveillance programme, the prevalence of *Salmonella enterica* in finisher herds is monitored serologically at slaughter, by use of a meat juice ELISA. Follow up in herds with a moderate or high number of seropositive pigs, as well as their suppliers, consists of mandatory bacteriological examinations of 20 pen samples. A pen sample is defined as a 25 g sample, consisting of 5 x 5 g faeces collected from the pen floor. Samples are analysed in the laboratory for presence of *S. enterica*. The aim of the present study was to investigate whether the cost of bacteriological examination at herd level could be reduced by cutting down on the number of analyses without loss of sensitivity.

Materials and Methods: The study was carried out in the laboratory between 1 May and 30 September 2002. Routine samplings collected as described above and sent to the lab for bacteriological examination were analysed by both the standard procedure (20 pen samples) and by mixing the same 20 samples into 10, 5 or 1 pooled sample(s), respectively. All bacteriological examinations were qualitative, carried out according to standard procedures, including non-selective pre-enrichment and selective enrichment. Serotyping of *Salmonella* isolates was only carried out for the original 20 pen samples.

Results: During the study period, 51 pig herds that submitted 20 pen samples to the laboratory were found *Salmonella*-positive (at least 1 positive sample) and were included in the study. First, all 51 herds were re-examined by pooling from 20 pen samples into 10. Subsequently, 25 herds were also examined by pooling from 20 pen samples into 5, whereas the remaining 26 herds were examined by pooling from 20 pen samples into 1. The results of the different pooling procedures are shown in Table 1. It can be seen that the relative sensitivity of the bacteriological analysis decreases moderately following pooling from 20 into 10 or from 20 into 5; and quite remarkably following pooling from 20 into 1. Due to the poor sensitivity observed, the results of pooling 20 samples into 1 were not analysed any further.

Table 1. Relative sensitivity of bacteriological analysis following examination of pooled faecal samples mixed according to different procedures.

| Procedure | Number of herds diagnosed Salmonella- positive* | Relative sensitivity (%) |
|-------------------|--|-----------------------------|
| 20 pooled into 10 | 48 / 51 | 94.1 |
| 20 pooled into 5 | 23 / 25 | 92.0 |
| 20 pooled into 1 | 19 / 26 | 73.1 |

* Compared to herds diagnosed Salmonella-positive by the standard procedure

Serotypes found in the examination of 20 pen samples included Typhimurium, Derby, Infantis, Worthington, Ohio, Yoruba and Livingstone.

On an individual sample basis, pooling of only Salmonella-negative samples, or only Salmonella-positive samples, gave good agreement between the standard procedure and pooled samples (Table 2). When the pooled sample was a mixture of negative and positive samples (in different ratios) from the original 20 pen samples, the percent agreement dropped to 61-66% (Table 2).

Table 2. Percent agreement of bacteriological results after pooling of all negative, mixed negative and positive, or all positive samples from the standard procedure.

| Combination of individual pen samples from standard procedure | Agreement (%) with standard procedure | |
|--|---------------------------------------|------------------|
| | 20 pooled into 10 | 20 pooled into 5 |
| All negative | 95.4 | 95.5 |
| Mixed (negative and positive) | 66.2* | 60.9* |
| All positive | 91.6 | 100.0 |

* Salmonella-positive result

Discussion and conclusion: Our results indicate that pooling 20 pen samples to either 10 or 5 samples in the laboratory will not seriously compromise the sensitivity of the bacteriological examination of *Salmonella*-suspect swineherds. It seems likely, as it is with the present standard procedure, that herds both with low and high *Salmonella* levels are detected using this approach. Herds with very low *Salmonella* levels may go undetected, but this is also true for the current procedure including analysis of 20 pen samples. Pooling 20 samples into a single sample led to a significant reduction in sensitivity, which is not acceptable with regard to food safety.

At individual sample level the agreement between the *Salmonella* result after pooling and the *Salmonella* result in the original samples was high when only *Salmonella*-negative samples or only *Salmonella*-positive samples were mixed. When positive samples were mixed with negative samples, agreement was still >60% for both pooling 20 samples into 10 and into 5. This suggests that the quantitative amount of *Salmonella* present in the original positive samples was sufficient to also give a positive result after pooling in more than 60% of cases.

The suggested alternative pooling methods should be carried out in the laboratory. Veterinarians should still collect 20 pen samples in the herd using the standard procedure, to ensure that a representative number of pigs are sampled from each herd.

O 02 Surveillance of zoonotic bacteria in finishing pigs in The Netherlands

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Summary: In The Netherlands, from 1998 till 2002, a surveillance programme for zoonotic bacteria in finishing pigs was conducted at herd level. In 2000-2002, the prevalence of *Salmonella* spp. approximated 30%, while a significantly decreasing trend was observed when standardizing data for herdsize, age and quarter of sampling. Serotype discrimination showed the predominance of *S. Typhimurium* with an increasing role for phage type DT104. Prevalence estimates for *Campylobacter* spp. were 97% in 1998 (4th quarter only) and 45% in 1999. For STEC O157, prevalence estimates were 2% and 0% in 1998 and 1999, respectively. By using the samples from this study, a comparison study was conducted in which three different selective enrichment media, i.e. RV, MSRV and DIASALM, were compared for the isolation of *Salmonella* spp. from pig feces. Both MSRV and DIASALM scored significantly better compared to RV. By using logistic regression analysis of farm and herd specific data, potential risk factors for *Salmonella* spp. in finishing pig herds were identified and quantified.

Introduction: *Salmonella* spp. and *Campylobacter* spp. are recognized world-wide as important zoonotic bacteria causing gastro-enteritis in humans. In 1999-2000, a study was conducted in the Dutch general population indicating a total number of cases of campylobacteriosis and salmonellosis of approximately 100,000 and 50,000, respectively (de Wit et al., 2001). Based on typing results, it has been estimated that about one quarter of the human infections with *Salmonella* spp. in The Netherlands results from the consumption of pork or pork products (Van Pelt et al., 1999). In order to provide critical information for the control of zoonotic bacteria in primary production, a national programme for surveillance of *Salmonella* spp., *Campylobacter* spp. and Shiga-toxin-producing *Escherichia coli* (STEC) O157 in farm animals was implemented in 1997 (Bouwknegt et al., 2003). The main objectives of this programme are to monitor trends in the occurrence of these zoonotic micro-organisms and to identify risk factors for infection of farm animals. Finishing pigs were included in the programme from October 1998.

Materials and Methods: A two-stage sampling scheme was used for estimation of prevalence at herd level. Each year, the primary sample size (number of herds to be sampled) was calculated for estimation of the *Salmonella* prevalence based on the expected prevalence, a desired accuracy of 5% and a confidence level of 90%. Pig farms were randomly selected from a national database stratified according to farm size and geographical region. On each farm, one herd (i.e. finishing pigs housed in the same barn, usually divided in several compartments) was randomly selected for sampling. Yearly, approximately 150 to 200 herds of finishing pigs were sampled. However, due to an

epidemic of Foot and Mouth disease, no samples were obtained from March 2001 till July 2001. Within a herd 12 to 60 samples were taken depending on the herd size, allowing by approximation the detection of $\geq 5\%$ shedding animals at a 95% confidence level. Fresh fecal samples were randomly collected from the floor and equally divided over 2 to 5 pooled samples. From 1998 till 2002, samples were examined for the presence of *Salmonella* spp. and additionally, in 1998 and 1999, the samples were examined for the presence of *Campylobacter* spp. and STEC O157. For the detection and typing of these micro-organisms, RIVM standard operating procedures were used. For the isolation of *Salmonella* spp., in 1998 and 1999, only Rappaport Vassiliadis (RV) was used as selective enrichment medium. In 2000 and 2001, in addition to RV, two semi-solid selective enrichment media, i.e. Modified semisolid RV (MSRV) and Diagnostic semisolid *Salmonella* medium (DIASALM), were used for comparison. In 2002, both RV and MSRV were used. In addition to the collection of microbiological data, at each farm visit, farm and herd specific information was collected in co-operation with the farm manager by using a questionnaire. Totally, data from 347 farms, collected in 2000 and 2001, were used in logistic regression analyses following the procedure of Hosmer and Lemeshow (1989).

Results: Figure 1 shows the crude annual prevalence estimates for *Salmonella* spp. in finishing pigs. No significant differences in crude estimates were observed between 2000 and 2002, approximating 30% in the last two years (based on the use of RV and MSRV). However, adjusting these crude estimates for the non-sampling period in 2001 resulted in a continuous decrease within this period. Trend analysis of the results standardized for herdsize, age and quarter of sampling, showed a significantly decreasing trend in these three years ($P=0.0343$). Serotype discrimination showed a two-fold increase in the prevalence of *S. Typhimurium* DT104 between 2000 and 2001. In 2002, a level comparable to 2001 was observed. Prevalence estimates for *Campylobacter* spp. were 97% in 1998 (4th quarter only) and 45% in 1999 with a predominance of *C. coli*. For STEC O157, prevalence estimates were 2% and 0%, respectively.

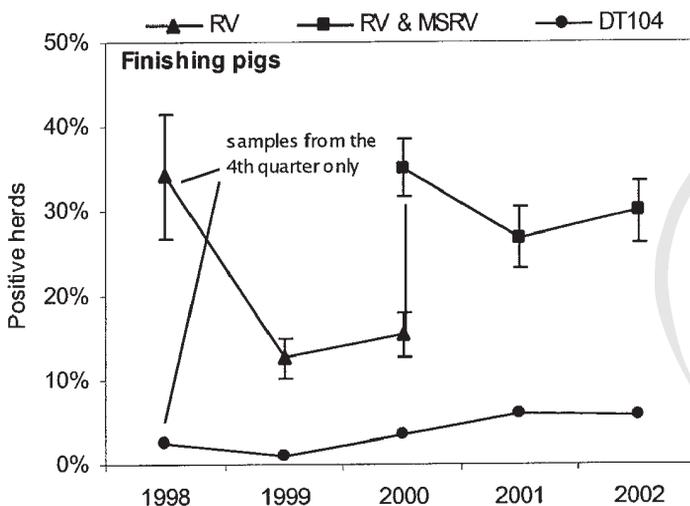


Figure 1. Crude annual prevalence estimates for *Salmonella* spp. in fattening pigs.

Table 1 shows the results of the comparison study for the isolation of *Salmonella* spp. from pig feces. Totally, 1591 samples were examined. Both MSRV and DIASALM scored significantly better than RV ($p < 0.01$). There was no significant difference between the results of MSRV and DIASALM. Combination

of two selective enrichment media yielded a higher number of positives in all cases. The combination of MSR/V and DIASALM scored significantly better than the combination of either MSR/V or DIASALM with RV ($p < 0.01$). The latter twocombinations did not differ significantly from each other. Multivariable logistic regression analysis (model based on 271 records) showed significant associations for: the seasons spring (Odds Ratio (OR) = 3.51; P value = 0.0164), summer (OR = 2.74; P = 0.0358) and autumn (OR = 3.76; P = 0.0077) all compared to winter, a moderate farm size (501-1000 animals) (OR = 4.22; P = 0.0032) compared to a small farm size (201-500 animals), one specific feed supplier (OR=12.3; P = 0.0027) compared to the reference supplier, the use of fermented liquid feed (OR=0.37; P = 0.0280) compared to its non-use, the use of natural ventilation (OR=6.18; P = 0.0231) compared to mechanical ventilation,

Table 1. Comparison of media for the isolation of *Salmonella* spp. from pig feces samples ($n = 1591$)

| Selective enrichment medium | no. of pos. samples after 24 h | % salm. Pos. after 24 h of total salm. pos. after 48 h | No. of pos. samples after 48 h | % salm. pos. after 48 h of total salm. pos. after 48 h |
|-----------------------------|--------------------------------|--|--------------------------------|--|
| RV | 69 | 36,9 | 82 | 43,9 |
| DIASALM | 108 | 57,8 | 151 | 80,7 |
| MSRV | 110 | 58,8 | 150 | 80,2 |
| RV + DIASALM | 131 | 70,1 | 161 | 86,1 |
| RV + MSRV | 125 | 66,8 | 168 | 89,8 |
| MSRV + DIASALM | 129 | 69 | 180 | 96,3 |
| RV + MSRV + DIASALM | 142 | 75,9 | 187 | 100 |

presence of a hygiene lock (OR=4.41; P = 0.0038) compared to its absence, presence of cats on the farm (OR=0.25; P = 0.0222) compared to the absence of animals and an age of 2-3 months (OR=0.17; P = 0.0446) compared to an age of 3-4 months.

Discussion: The Dutch national surveillance programme in finishing pigs yielded valuable information on the occurrence of zoonotic bacteria, the suitability of isolation methods for *Salmonella* spp. and risk factors for *Salmonella* infection.

The use of an additional, more sensitive selective enrichment medium (MSRV) resulted in two-fold higher prevalence estimates. In 2000-2002, the prevalence of *Salmonella* spp. approximated 30%, while a significantly decreasing trend was observed when standardizing data for herdsize, age and quarter of sampling. The latter finding may be due to an increased awareness of good hygiene practices in the pig primary production sector. Serotype discrimination showed the predominance of *S. Typhimurium* with an increasing role for phage type DT104, which corresponds with an increase of this specific phagetype in human salmonellosis (van Pelt, 2001). The prevalences measured for *Campylobacter* spp. indicate that pigs are an important reservoir for this micro-organism. However, since the main species was *C. coli* and *Campylobacter* contamination of pork is normally very low (van der Zee et al., 2000), the relative importance of pork in the epidemiology of campylobacteriosis is likely to be small. Furthermore, pigs do not seem to be an important reservoir for STEC O157.

The results of the method comparison study clearly indicate that the semi-solid media MSR/V and DIASALM are more sensitive for detection of *Salmonella* spp. in pig feces compared to the liquid RV medium. Similar results have been found for poultry feces (Voogt et al., 2001). However, since the isolation principle of these semi-solid media is based on migration of the bacteria through the agar,

these media are not appropriate for detection of non-motile or less motile *Salmonella* bacteria. Therefore, a combination of a semi-solid medium with a liquid medium, such as RV, is recommended. Several factors were significantly associated with the presence of *Salmonella* spp. The increased risk associated with natural ventilation might be related to biosecurity, which is likely to be lower in naturally ventilated barns. The decreased risk associated with an age of 2-3 months compared to an age of 3-4 months might be related to one or more causes, *i.e.* (i) maternal immunity preventing *Salmonella* colonisation at a younger age, (ii) a preventive effect of medication administered at an early stage of life, or (iii) enhanced exposure to *Salmonella* after transportation to a finishing barn (usually around 10 weeks of age). The protective effect found for the use of fermented liquid feed is hypothetically related to the presence of organic acids in this type of feed which may inhibit the presence of *Salmonella* in the feed and enhance the acid barrier of the stomach (van der Wolf et al., 2001). The preventive effect found for the presence of cats on the premise might be related to the expelling of rodents, which are considered important vectors for *Salmonella* infection (Lo Fo Wong et al., 2002). For the other associations determined, no hypotheses could be generated.

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Campylobacter prevalence in Danish finisher pigs from mixed production herds

O 03

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Summary: The prevalence of *Campylobacter jejuni* was investigated in finisher pigs from 89 herds with pigs and cattle, 68 with pigs and poultry, and 90 with pigs only. *C. jejuni* was detected in pigs from 8.5% of herds examined. There was no significant difference in *C. jejuni* prevalence between herd types. Verification of 5 additional colonies from each *C. jejuni*-positive sample yielded *C. jejuni* only in 50% of samples, while the remaining 50% were mixed infections. The results suggest that *C.*

jejuni prevalence in Danish finisher herds is low. However, herds with high levels of *C. jejuni* in pigs do exist, and individual pigs may excrete high *C. jejuni* levels.

Keywords: bacteriological screening, swine, cattle, poultry, risk factor.

Introduction: In Denmark, 4,379 cases of human *Campylobacter* infection were registered in 2002. More than 90% were caused by *C. jejuni*, of which the majority were ascribed to poultry meat. Even though pigs are known to harbour high levels of *C. coli*, this is not reflected in the human incidence. However, *C. jejuni* is detected occasionally in slaughter pigs and therefore a possible role for pigs in transmission to humans cannot be excluded. Particularly pigs raised in mixed production systems involving *C. jejuni*-infected poultry or cattle could be at risk of becoming infected. The aim of our study was to investigate the prevalence of *C. jejuni* in finisher pigs from mixed production herds, compared to herds producing only pigs.

Materials and Methods: We conducted a screening for *Campylobacter* spp. in Danish finisher herds. Herds were stratified into 3 types: (1) pigs and cattle (dairy or beef), (2) pigs and poultry (broilers or laying hens), and (3) pigs only. We hypothesised that *C. jejuni* prevalence in pigs raised in mixed production herds would be significantly higher compared to herds producing only pigs. Cecal samples from 5 randomly chosen finisher pigs per herd were collected at the slaughterhouse and analysed for presence of *C. coli* and *C. jejuni* using standard bacteriological culture and typing methods.

Results: Cecal samples were collected from 247 herds: 89 herds with cattle and pigs, 68 with poultry and pigs, and 90 with pigs only. *Campylobacter* was found in all herds. The prevalence of *C. coli* in pigs was 90.1%, while *C. jejuni* was found in 2.3% of pigs. *C. jejuni* was found in at least 1 sample in 21 of 247 herds (8.5%), but there was no statistical difference ($p = 0.22$) in *C. jejuni* prevalence between herd types (Table 1).

Table 1. Prevalence of *Campylobacter jejuni* in Danish finishing pigs according to herd type. Five cecal samples were collected at the slaughterhouse from each of 247 herds, and analysed for *C. jejuni*.

| Herd type | Number of herds examined | <i>C. jejuni</i> -positive herds | | |
|------------------|--------------------------|----------------------------------|------------|---------------------|
| | | N | % | 95% C.I. |
| Pigs and cattle | 89 | 11 | 12.4 | 5.55 – 19.25 |
| Pigs and poultry | 68 | 3 | 4.4 | 0.0 – 9.27 |
| Pigs | 90 | 7 | 7.8 | 2.26 – 13.34 |
| Total | 247 | 21 | 8.5 | 5.02 – 11.98 |

In 5 of 21 *C. jejuni*-positive herds, multiple samples yielded *C. jejuni*. Verification of 5 additional isolates from 24 *C. jejuni*-positive samples yielded only *C. jejuni* in 50% of cases, while an additional 25% showed mixed infection with both *Campylobacter* species. For the remaining 25%, all extra colonies were typed as *C. coli* (Table 2).

Table 2. Verification of 5 additional colonies from 24 *C. jejuni*-positive caecal samples collected from Danish slaughter pigs.

| Herd type | Number of <i>C. jejuni</i> isolates | Verification using 5 extra isolates from the same sample | | |
|------------------|-------------------------------------|--|------------------------------|---|
| | | Isolates only <i>C. jejuni</i> | Isolates only <i>C. coli</i> | Isolates both <i>C. jejuni</i> and <i>C. coli</i> |
| Pigs and cattle | 14 | 6 | 3 | 5 |
| Pigs and poultry | 3 | 3 | 0 | 0 |
| Pigs | 7 | 3 | 3 | 1 |
| Total | 24 | 12 (50%) | 6 (25%) | 6 (25%) |

Based on the observed overall *C. jejuni* prevalence of 2.3% and the assumption that *C. jejuni* was distributed randomly in the study population, the probability of detecting more than 1 *C. jejuni* positive caecal sample per herd in the present study could be calculated using a binomial distribution (Bin (5, 0.023)). The expected and observed distributions of *C. jejuni* positive herds in the study population (N=247) are shown in Table 3. The number of herds with more than 1 *C. jejuni* positive sample is significantly greater ($p=0.01$) than could be expected.

Table 3. Probability for detection of *C. jejuni* positive caecal samples in 5 finisher pigs per herd in the present study, based on a binomial distribution Bin (5, 0.023).

| Number of <i>C. jejuni</i> positive caecal samples per herd | Probability | Distribution of 247 herds with respect to number of <i>C. jejuni</i> positives out of 5 samples | |
|---|-------------|---|-----------------|
| | | Expected number | Observed number |
| 0/5 | 0.8889 | 220 | 226 |
| 1/5 | 0.1060 | 26 | 16 |
| 2/5 | 0.0051 | 1 | 3 |
| 3 or more | 0.0001 | 0 | 2 |

Discussion and conclusion: Our study shows that 100% of swine herds examined were *Campylobacter*-positive. Mixed production type herds did not have significantly higher *C. jejuni* prevalences compared to herds with only pig production. However, the relatively low occurrence of *C. jejuni* in herds with pigs and poultry may indicate that more effective transmission barriers are in place in these herds, in contrast to the more open structure of herds also producing cattle. This should be investigated further at herd level, including *C. jejuni* prevalences in cattle and poultry in mixed production herds.

Our results suggest that the overall *C. jejuni* prevalence in Danish finishers is low. However, the prevalence, and hence the probability of detection, of *C. jejuni* is higher in certain herds in the present study, suggesting that herds with high levels of *C. jejuni* in pigs do exist. Our results furthermore indicate that individual pigs may excrete *C. jejuni* levels that are higher than *C. coli* levels. Further studies must clarify herd risk factors for *C. jejuni* in pigs, as well as possible differences in survival of *C. jejuni* and *C. coli* on carcasses.

O 04

Eliminating the Abattoir Pen Lairages to Decrease the Prevalence of Salmonella in Cull Sows

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Summary: The study objective, to determine the role of abattoir antemortem pens in preharvest *Salmonella enterica* contamination, was conducted over 4 sampling periods, February-April 2002. A total of 40 sows were selected for each period at the same collection point and transported to the abattoir. Twenty (20) were unloaded and sent directly to harvest and 20 held in antemortem pen for 2 h before harvest. Samples collected included ileocecal and subiliac lymph nodes, cecal and transverse colon contents, pre-rinse carcass sponge swabs for the right and left carcass sections and chopped meat blocks composited from these carcasses. The percentage of positive samples (all tissues) and cecal content from sows held in the antemortem pens (59%, 55 %, respectively) were significantly higher ($P < 0.05$) compared to direct delivered (44%, 39 % respectively). This study demonstrates that normal antemortem holding practices contributed to increased *Salmonella enterica* contamination of the digestive tract.

Keywords: food safety, zoonosis, antemortem handling, swine, meat science

Introduction: *Salmonella enterica* contamination for all pork product classes is a growing worldwide concern. We have previously demonstrated that 40-70 % of Midwestern USA market swine are *Salmonella* positive after normal farm-direct transportation and antemortem holding (McKean et al, 2001). Cull sows may present a greater potential risk for introduction of food-borne *Salmonella* contamination, in part because of accepted centralized collection, holding, and handling practices with animal commingling, and foods produced. They may become infected with *Salmonella* either directly by contaminated feed or water, or indirectly by exposure to a contaminated peri-marketing environment. Much of the pork derived from cull sows consists of ground meats used in processed products (pepperoni, salami, etc) sold directly to consumers. Therefore, *Salmonella* contamination may be a greater food-borne risk in pork derived from cull sows. Few studies have examined *Salmonella* risk relationships under modern procurement and processing practices. This study was designed to examine the role of the abattoir antemortem pen in peri-marketing *Salmonella* contamination.

Materials and Methods: Four sampling periods, from February–April 2002, involved 160 cull sows handled under normal marketing conditions. Buying station personnel selected cull sows from a larger population from a common production source. Sows ($n = 40$) selected each period were transported to the abattoir by commercial trucker, where 20 sows were unloaded and sent directly to harvest. The other 20 sows were held in antemortem pens for 2 h and harvested under the same conditions. Prior to entry of sows four 100 cm² 4-ply gauze squares were placed on the pen floor. The postmortem collection of transverse colon contents was substituted for an antemortem fecal sample because of safety concerns associated with obtaining a pre-harvest sample in the available antemortem pen environment.

Each carcass was individually identified. Samples collected included ileocecal lymph node (5-10 g), subiliac lymph node (1-5 g), cecal contents (20 g), transverse colon contents (15 g), pre-carcass wash sponge swabs of the left and right carcass section, utilizing the standard three-site USDA-FSIS procedure (300 cm²), and chopped meat block samples from a mixture of each 20 sow group. All samples were placed in whirl-pak bags and transported on ice to the National Animal Disease Center, Ames, Iowa.

ORAL PRESENTATIONS

Samples were handled in the following manner. Colon (10 g) and cecal (10 g) contents each were placed in 90 ml of tetrathionate and of GN-Hajna broths, two enrichment media. The subiliac lymph nodes from each carcass side were pooled, placed in a sterile bag, and macerated with a rubber mallet. The ileocecal lymph nodes were treated in the same manner. Buffered peptone water (10 ml) was added to each lymph node-containing bag and the sample was homogenized for one minute in a stomacher at 260 rpm. One ml of supernatant was added to 9 ml for each of the two enrichment media. Carcass (separate for each side) and environmental sponges were cut lengthwise into four sections and placed into 18 ml of each enrichment medium. All inoculated media were incubated overnight at 37 °C. At 24 h, the tetrathionate and GN-Hajna cultures were transferred (1:100) into Rappaport R-10 medium and incubated 24 h at 37 °C. After 24 h, all Rappaport enrichments were screened with an enzyme-linked immunosorbent assay (ELISA). (Rostagno et al, 2001). Samples with $OD_{\lambda} > 0.400$ were considered positive and were streaked onto xylosine-lysine-tergitol-4-agar (XLT-4) plates and incubated overnight (37 °C). One colony from each plate exhibiting typical *S. enterica* appearances was picked to Rambach agar and incubated overnight (37 °C). Presumptive positive isolates were serogrouped and picked to tryptase soy agar (TSA) slants, then submitted to the National Veterinary Services Laboratory, Ames, Iowa, for serotype determination.

Results: Sows (n = 160) were sampled over 12 weeks. Selected animals were handled identically except for the 2 h antemortem holding period. Individual animal results were collected and analyzed, but combined for reporting purposes. Table 1 summarizes the data for all Salmonella isolations. Overall, there was a statistically significant difference for recovery of Salmonella from cecal contents ($P < 0.03$) and from overall carcass samples ($P < 0.05$) when treatment (hold) and control groups (no hold) were aggregated and compared. All other sample types were not influenced by holding. Table 2 summarizes isolate serotypes from antemortem pens and hold and no-hold animals.

Table 1. Tissue percentage positive samples and number tested of sows held for two hours vs. no hold.

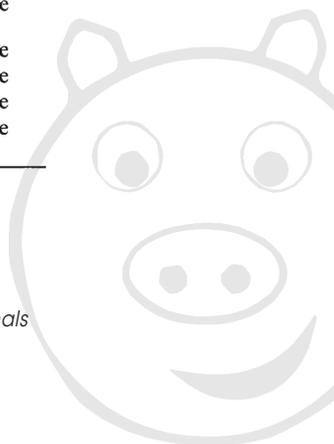
| | Holding | No Holding | -P-value |
|----------------------|-----------|------------|---------------|
| Transverse Colon | 5 % (80) | 4 % (80) | No Difference |
| Cecal Contents | 39 % (80) | 55 % (80) | $P < 0.03$ |
| Ileocecal Lymph Node | 9 % (80) | 9 % (80) | No Difference |
| Subiliac Lymph Node | 0 % (80) | 1 % (80) | No Difference |
| Carcass Swabs | 1 % (80) | 0 % (80) | No Difference |
| Meat Block * | 0 % (7) | 0 % (80) | No Difference |
| Carcass Overall # | 44 % (80) | 59 % (80) | $P < 0.05$ |

* Consisted of twenty sows sub sampled seven times.

Consisted of transverse colon, cecal contents, ileocecal and subiliac lymph nodes

Table 2. Aggregated serotypes isolated from pens and animals

| Serovar | Pen | No Hold | Hold |
|--------------------|-----|---------|------|
| 6.7:z10-monophasic | | 3 | 10 |
| Bovis-Morbicans | 1 | | |
| Brandenburg | | 16 | 6 |
| Derby | 4 | 12 | 19 |
| Hadar | | 8 | 4 |
| Infantis | | 6 | 5 |
| Johannesburg | | 3 | 2 |
| Manhattan | 2 | | |
| Reading | 6 | | |
| Senftenberg | 1 | | 15 |
| Tennessee | | 1 | |
| Typhimurium | | 1 | |
| Uganda | 2 | | |



Discussion: The 2 h antemortem pen holding period was sufficient to influence the isolation rates in cecal contents but not for other tissues sampled. The recovery of *Salmonella* from cecal contents increased more quickly than other tissues when sows were exposed to contaminated environments of the holding pen. This observation is consistent with reports in finishing swine. The level of cecal contamination was higher in sows resting in the holding pens (55 %) than in animals harvested directly upon arrival at the abattoir, although not as large a difference as observed in other studies. The heightened entry contamination level may be caused by several interrelated factors. The cull sows had 18 - 24 h of exposure to an environment (collection point) where commingling with sows of other farms occurred. Therefore, both direct and indirect exposure opportunities were possible. These indirect routes from farms to abattoir and continuous exposures to commingled environments offered opportunities for contamination prior to abattoir delivery. The potential for increasing the cecal isolation rates significantly over a 2-h holding period is decreased as the entering baseline (no-hold) percentage rises. This factor may partially explain the absence of significant differences in cecal content and other tissue isolations when individual test periods are analyzed, but a significant difference in cecal contents when overall recoveries are compared. In addition, weekly variations in the levels of antemortem pen environmental contamination may have contributed to this difference.

The much lower isolation rates for the transverse colon as compared to the cecal contents and the lack of difference in isolation rates between direct and holding groups for other tissues were not anticipated. This observation is consistent with a transient infection that has not had sufficient time to traverse the entire tract. The relatively low ileocecal lymph node isolation rates within and between groups may result from a similar temporal or dose exposure function. Low-level environmental exposures may be sufficient to contaminate cecal contents but inadequate to consistently be taken into the gut-associated lymph system. Additional work is needed to further elucidate this observation and relative epidemiologic importance.

Finding minimal *Salmonella* isolations from the carcass-associated lymph system, as represented by the subiliac lymph nodes, is consistent with market swine observations. As with the ileocecal nodes, there may be a threshold exposure level necessary for colonization. The minimal carcass surface (carcass swabs) and meat block contamination rates most likely represent the results of Hazard Analysis Critical Control Points (HACCP) controls implemented by the processing facility. Even though a substantial percentage of the gut tracts contained *Salmonella* at harvest, it was not transferred from the gut to the carcasses during processing. The greater serotype diversity and frequency of isolation in the "hold" groups indicated that the antemortem pen was a likely contributor to increased *Salmonella* isolation rates. Because only four of the seven pen isolates were recovered in later cultures, this may reflect bias introduced in selecting colonies for further serotyping. Whether they were represented on the initial culture plate and not selected, cannot be determined under current procedures. This study demonstrates that normal antemortem holding practices contributed to increased *Salmonella enterica* contamination of the digestive tract at harvest.

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APPEARANCE OF MULTIRESTANT *SALMONELLA* TYPHIMURIUM DT104 IN SWINE IN POLAND

O 05

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Summary: *Salmonella* (S.) Typhimurium phage type distribution remains unrecognised in Poland. We describe DT104 that appeared in swine no later than in 1998. The study revealed the presence of two integrons of 1.0 kb and 1.2 kb referring to, aminoglycoside and b-lactamase resistance genes cassette respectively in all DT104 and in one NT strain showing ACSSuT profile. These isolates tend to demonstrate additional resistance profiles. The findings illustrate the world-wide spread of multiresistant clone and point out the import of subclinically infected animals as a source of DT104 introduction into Polish swine herds.

Keywords: pigs, salmonellosis, antibiotic resistance, integrons

Materials and Methods: The study included *S. Typhimurium* strains isolated during 1994-2002 (n=20) and 1979-1988 (n=8) from clinical samples of diseased pigs. Four of the isolates originated from imported animals. MIC was determined with microbroth two-fold dilution method (Sensititre) and interpreted according NCCLS guidelines. The strains were phagetyped according to Callow's system (Anderson E.S. et al., 1977). Integron detection by PCR using the primers att1-F and 3_CS-B, were performed as described by Sandvang et al. (Sandvang D, Diggle M Platt DJ, 2002). Contingency test (Yates's correction) at the 0.05 level of significance was used for statistical purposes.

Results: *S. Typhimurium* characteristics were gathered in table 1. No apramycin, gentamicin, colistin, ceftiofur, amoxicillin/ clavulanic acid and ciprofloxacin resistance was found. Nine isolates were sensitive to all antimicrobials tested and belonged to 7 different phage types or showed unspecific reactions (RDNC). Resistance was more frequently found in isolates from 1994-2002 ($p \leq 0.01$) and DT110 and DT104 predominated amongst five phage types detected. Tetracycline-resistance as a single resistance determinant was observed exclusively in DT110 and DT208 isolates ($p \leq 0.01$). Two DT110 and 1 NT strain showed ASSuT profile. ACSSuT resistance was observed in 6 DT104 and 1 NT strain that revealed also the presence of PCR products corresponding to the resistance gene cassette.

Discussion: *S. Typhimurium* is either the prevalent or the second *Salmonella* serovar most frequently found in swine (Baggesen, D. L., Sandvang, D., and Aarestrup, F. M., 2000; Hoszowski A. and Wasyl D., 2002). Despite the importance of *S. Typhimurium* as a cause of swine infections, the phage type distribution in Poland remains unknown. Therefore the aim of the research was to confirm the appearance of *S. Typhimurium* DT104 in swine in Poland and determine the presence of class 1 integrons. DT104 has not yet been reported in Poland and this paper should be considered as the first report on this phage type with integron mediated multi-resistance in animals. The first strain was obtained in 1998 and since then DT104 has been isolated from swine salmonellosis. It was found in imported animals introduced into breeding herds. All ACSSuT strains revealed the presence of 1.0 kb and 1.2 kb PCR products corresponding to two common integrons of the resistance gene cassettes (Baggesen, D. L., Sandvang, D., and Aarestrup, F. M., 2000; Lawson, A. J. et al., 2002; Sandvang D, Diggle M Platt DJ, 2002). Integron positive nontypable strain was due to decreased phage sensitivity rather than appearance of multi-resistance genes in non-DT104 isolates (Lawson, A. J., Dassama, M. U., Ward, L. R., and Threlfall, E. J., 2002). Integron-positive *S. Typhimurium* strains had additional resistance determinants (Baggesen, D. L., Sandvang, D., and Aarestrup, F. M., 2000). Resistance profiles noted in present study

gathered up to 10 antimicrobials. DT104 strains were often nalidixic acid resistant ($p \leq 0.001$). Although florfenicol, neomycin and trimethoprim joined the basic penta-resistant profile, no significance was noted in respect of those antimicrobials. The findings illustrate the world-wide clonal spread of multi-resistant *S. Typhimurium* DT104.

Conclusions: Penta-resistant *S. Typhimurium* DT104 has been present in Polish swineherds since at least 1998. The possible source of introduction of the clone to Poland was imported symptomless, chronically infected animals.

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Table 1. *S. Typhimurium* antibiotic susceptibility, R-types [-] – susceptible strain), phage types and presence of integrons (n – not detected, p – detected)

| Strain No. | Year | MIC (µg/ml) | | | | | | | | | | | | | | | | R-types | Phage types | Integrons | |
|------------|------|-------------|----|-----|-----|----|------|-----|----|-----|-----|----|-----|----|-----|----|----|---------|------------------|-----------|-----|
| | | A/C | A | Apr | Cft | C | Cip | Col | Ff | Gen | Na | N | Sp | S | Su | T | Tm | | | | T/S |
| 2416 | 1979 | 2 | 1 | 4 | 0,5 | 4 | 0,03 | 4 | 2 | 1 | 4 | 2 | 32 | 8 | 32 | 2 | 4 | 1 | [-] | DT66 | n |
| 2466 | 1979 | 2 | 1 | 4 | 0,5 | 2 | 0,03 | 4 | 4 | 1 | 4 | 2 | 128 | 32 | 512 | 32 | 32 | 8 | SSuTSpt/STm | DT135 | n |
| 2468 | 1979 | 2 | 1 | 4 | 0,5 | 8 | 0,03 | 4 | 4 | 1 | 4 | 2 | 32 | 8 | 32 | 32 | 4 | 1 | T | DT110 | n |
| 15 | 1980 | 2 | 1 | 4 | 0,5 | 4 | 0,03 | 4 | 2 | 1 | 8 | 2 | 32 | 16 | 32 | 2 | 4 | 1 | [-] | DT37 | n |
| 471 | 1985 | 2 | 1 | 4 | 0,5 | 4 | 0,03 | 4 | 2 | 1 | 4 | 2 | 32 | 8 | 64 | 2 | 4 | 1 | [-] | RDNC | n |
| 501 | 1985 | 2 | 1 | 4 | 0,5 | 4 | 0,03 | 4 | 4 | 1 | 8 | 2 | 32 | 8 | 32 | 2 | 4 | 1 | [-] | U275 | n |
| 592 | 1985 | 2 | 1 | 4 | 0,5 | 2 | 0,03 | 4 | 2 | 1 | 4 | 2 | 16 | 8 | 32 | 2 | 4 | 1 | [-] | DT9 | n |
| 92 | 1988 | 2 | 1 | 4 | 0,5 | 2 | 0,03 | 4 | 2 | 1 | 4 | 2 | 32 | 4 | 32 | 2 | 4 | 1 | [-] | DT135 | n |
| 491 | 1994 | 2 | 2 | 4 | 0,5 | 4 | 0,03 | 4 | 2 | 1 | 4 | 2 | 32 | 8 | 32 | 2 | 4 | 1 | [-] | RDNC | n |
| 606 | 1996 | 2 | 2 | 4 | 0,5 | 4 | 0,03 | 4 | 4 | 1 | 4 | 2 | 32 | 8 | 32 | 32 | 4 | 1 | T | DT208 | n |
| 607 | 1996 | 2 | 2 | 4 | 0,5 | 4 | 0,03 | 4 | 4 | 1 | 4 | 2 | 32 | 8 | 32 | 32 | 4 | 1 | T | DT208 | n |
| 609 | 1996 | 2 | 2 | 4 | 1 | 4 | 0,03 | 4 | 4 | 1 | 4 | 2 | 32 | 8 | 32 | 32 | 4 | 1 | T | DT208 | n |
| 118 | 1998 | 16 | 32 | 4 | 0,5 | 64 | 0,03 | 4 | 16 | 1 | 4 | 2 | 128 | 64 | 512 | 16 | 4 | 1 | ACSSuTSp | DT104 | p |
| 1102 | 2000 | 2 | 1 | 4 | 0,5 | 4 | 0,03 | 4 | 4 | 1 | 4 | 2 | 32 | 8 | 32 | 2 | 4 | 1 | [-] | DT41 | n |
| 1103 | 2000 | 8 | 32 | 4 | 0,5 | 8 | 0,03 | 4 | 4 | 1 | 4 | 2 | 32 | 64 | 512 | 32 | 4 | 1 | ASSuT | DT110 | n |
| 216 | 2000 | 16 | 32 | 4 | 1 | 64 | 0,25 | 4 | 32 | 1 | 128 | 2 | 128 | 64 | 512 | 16 | 4 | 1 | ACSSuTSpNaFf | DT104 | p |
| 425 | 2000 | 4 | 32 | 4 | 0,5 | 8 | 0,03 | 4 | 4 | 1 | 8 | 2 | 32 | 64 | 512 | 32 | 4 | 1 | ASSuT | NT | n |
| 816 | 2000 | 16 | 32 | 4 | 0,5 | 64 | 0,25 | 4 | 16 | 1 | 128 | 2 | 128 | 32 | 512 | 32 | 4 | 1 | ACSSuTSpNa | DT104 | p |
| 190 | 2001 | 16 | 32 | 4 | 1 | 64 | 0,03 | 4 | 32 | 1 | 4 | 2 | 128 | 64 | 512 | 32 | 4 | 1 | ACSSuTSpFf | NT | p |
| 191 | 2001 | 2 | 1 | 4 | 0,5 | 4 | 0,03 | 4 | 4 | 1 | 4 | 2 | 32 | 8 | 32 | 32 | 4 | 1 | T | DT110 | n |
| 192 | 2001 | 2 | 1 | 4 | 0,5 | 8 | 0,03 | 4 | 4 | 1 | 4 | 2 | 32 | 8 | 32 | 32 | 4 | 1 | T | DT110 | n |
| 193 | 2001 | 2 | 1 | 4 | 1 | 8 | 0,03 | 4 | 4 | 1 | 4 | 2 | 32 | 8 | 32 | 32 | 4 | 1 | T | DT110 | n |
| 194 | 2001 | 2 | 1 | 4 | 0,5 | 8 | 0,03 | 4 | 4 | 1 | 4 | 2 | 32 | 8 | 32 | 32 | 4 | 1 | T | DT110 | n |
| 215 | 2001 | 8 | 32 | 4 | 0,5 | 8 | 0,03 | 4 | 4 | 1 | 4 | 2 | 32 | 64 | 512 | 32 | 4 | 1 | ASSuT | DT110 | n |
| 298 | 2001 | 16 | 32 | 4 | 0,5 | 64 | 0,25 | 4 | 64 | 1 | 128 | 2 | 128 | 64 | 512 | 32 | 4 | 1 | ACSSuTSpNaFf | DT104 | p |
| 323 | 2001 | 16 | 32 | 4 | 0,5 | 64 | 0,25 | 4 | 16 | 1 | 128 | 32 | 128 | 64 | 512 | 32 | 32 | 8 | ACSSuTNSpNaT/STm | DT104 | p |
| 618 | 2001 | 2 | 1 | 4 | 0,5 | 4 | 0,03 | 4 | 2 | 1 | 8 | 2 | 32 | 16 | 32 | 2 | 4 | 1 | [-] | DT8 | n |
| 7 | 2002 | 16 | 32 | 4 | 0,5 | 64 | 0,25 | 4 | 16 | 1 | 128 | 2 | 128 | 32 | 512 | 16 | 4 | 1 | ACSSuTSpNa | DT104 | p |

Molecular Epidemiology of *Salmonella enterica* and Subtyping Using Phenotypic and Genotypic Approaches

O 06

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Summary: The aim of this study was to evaluate the discriminatory power of two phenotyping and three genotyping methods commonly used to subtype *Salmonella* in swine and other hosts. We found AFLP and PFGE to have the highest and comparable discriminatory power to each other. Among the 202 isolates analyzed in this study, using AFLP, 16 cluster types of *S. Typhimurium* were identified. Vertical spread in the production chain, from nursery to finishing farms and vertical as well as horizontal spread among finishing farms appeared to be important means of *Salmonella* serovar Typhimurium dissemination in swine units.

Keywords: fingerprint, food safety, salmonellae, phage type, antibiotic resistance

Introduction: Diversity among salmonellae has been studied using various phenotypic methods including serotyping, antibiotyping and phage typing, and by genotypic methods including pulsed field gel electrophoresis (PFGE) (Schwartz and Cantor, 1984), amplified fragment length polymorphism (AFLP), plasmid profiling, ribotyping, and other related methods (Gebreyes, 2003). Genotyping of *Salmonella* serovars is becoming an increasingly important epidemiological tool that aids in identifying sources of infection during outbreaks, detecting cross transmissions, recognizing particular strains, and monitoring intervention strategies. The aim of this study was to evaluate the discriminatory power of two phenotyping and three genotyping methods and to recommend standard approaches for epidemiological applications.

Materials and Methods: All 202 isolates of *Salmonella enterica* serovar Typhimurium included in this study originated from the longitudinal study described previously (Funk et al., 2001). Antibiotyping was done using two methods: Vitek Jr. (Biomérieux, Hazelwood, MO) and Kirby-Bauer disk diffusion methods as recommended by the National Committee for Clinical Laboratory Standards. Serotyping and phage typing was performed by the National Veterinary Services Laboratories (NVSL) in Ames, Iowa. PFGE, AFLP and repetitive extragenic palindromic PCR (Rep-PCR) genotyping methods were used as described previously (Gebreyes, 2003). DNA fingerprints were analyzed using the Bionumerics software (Applied Maths, Kortrijk, Belgium). We applied Simpson's index of diversity (DI) to compare the discriminatory power of the five subtyping systems used in this study.

Results: We analyzed the discriminatory power of the five subtyping methods (AFLP, PFGE, Rep-PCR, antibiotyping and phage typing) to characterize 202 isolates. We found AFLP and PFGE to have the highest and comparable discriminatory power with a DI value of 0.939 and 0.925 respectively (Table 1). The lowest discriminatory power was observed by the Rep-PCR technique. The latter technique did not appear to discriminate within serovar when using the universal primers (UPRIME-B1) recommended by the manufacturer. As demonstrated in figure 1-A, using the Rep-PCR approach, phenotypically distinct isolates (with different resistance pattern and phage types) appeared to have similar genotypes. In contrast, as demonstrated in figure 1-B and 1-C, AFLP and PFGE were able to discriminate within distinct phenotypes of serovar Typhimurium (Figure 1). Using the standards proposed previously (Gebreyes, 2003), among the 202 isolates analyzed in this study, 16 cluster types were identified by using AFLP. Vertical spread in the production chain, from nursery to finishing farms

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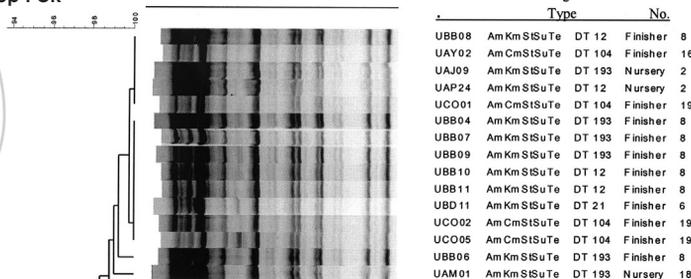
and vertical as well as horizontal spread from nursery and among finishing farms appeared to be important means of *Salmonella* serovar Typhimurium dissemination in swine production units.

Table 1. Subtyping methods included in this study and discriminatory index values of each.

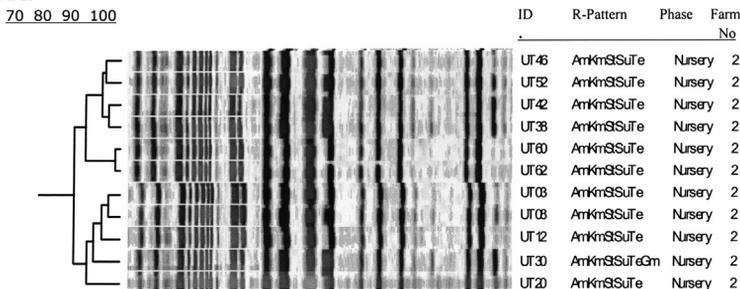
| Subtyping Method | No. of types | Size (%) of Largest type | Discriminatory index (DI) |
|------------------|----------------------|--------------------------|---------------------------|
| AFLP | 16 | 12 | 0.939 |
| PFGE | Performed on subsets | | 0.925 |
| REP-PCR | Performed on subsets | | 0.421 |
| Antibiotyping | 12 | 56 | 0.579 |
| Phage typing | 9 | 55 | 0.628 |

Figure 1. Illustrations of genotyping of *Salmonella enterica* serovar Typhimurium isolates using the three genotyping methods described. 1-A. Rep-PCR; 1-B. AFLP and 1-C. PFGE (Reprinted with permission from the *Journal of Swine Health and Production*, American Association of Swine Veterinarians).

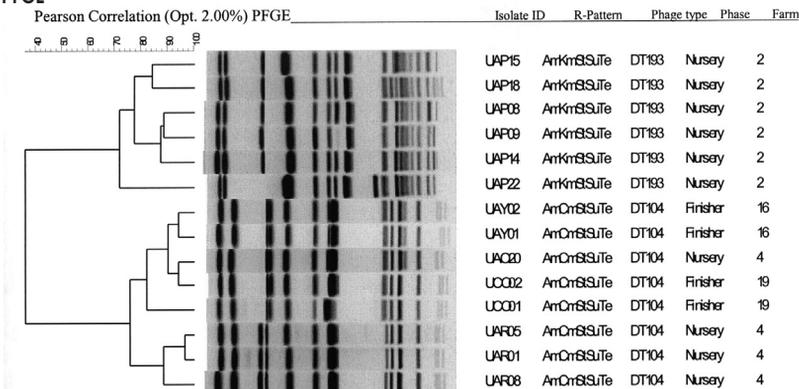
1-A. Rep-PCR



1-B. AFLP



1-C. PFGE



Discussion and Conclusions: Among genotyping methods, PFGE has been shown to have superior discriminatory power and reproducibility. Currently, it is the gold standard fingerprinting technique used for subtyping foodborne bacterial pathogens in humans under the pulsenet system (CDC). AFLP has also, in recent years, gained popularity due to its high discriminatory power and reproducibility. However, these methods are also known to have a high initial cost and moderate ease of use (Gebreyes, 2003). Based on phenotypic classification of isolates in the present study, the most common and widespread cluster type (cluster type 11) was composed predominantly of phage type DT104. The genetic diversity within DT104 in the present study was limited as compared to other phage types. This is consistent with previous reports. Using antibiotyping as an alternative approach, two predominant pentaresistance types among 12 antibiotypes have been detected: AmCmStSuTe and AmKmStSuTe patterns (Gebreyes and Altier, 2002). The use of phage typing in addition to genotypic approaches remains an important step in subtyping *Salmonella*, particularly in outbreaks involving serovars Typhimurium and Enteritidis as recommended previously (Ward et al., 2001).

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***Campylobacter* Prevalence and Diversity in Antimicrobial Free and Conventionally Reared Market Swine**

O 07

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Summary: The objectives of this study were to determine the prevalence and antimicrobial resistance of *Campylobacter spp.* among pigs raised antimicrobial free (ABF) and those raised conventionally. Bacterial isolation was done on-farm and at slaughter using conventional methods and antimicrobial susceptibility tests were done for 12 antimicrobials using Kirby-Bauer and epsilon-metric test (E-test) methods. All 14 herds were positive for *Campylobacter*. On-farm prevalence among ABF herds was 71% and 81% among conventional herd. In contrast, the prevalence among carcass swabs was higher among ABF herds than conventional herds with 60% and 29% respectively. There was significant reduction after chilling in all groups ($p < 0.05$). On-farm frequency of antimicrobial resistance was significantly higher among isolates from conventional herds than ABF ($p < 0.05$). In contrast frequency of resistance to five of the seven antimicrobials was higher among carcass swabs of ABF herds than conventional herds.

Introduction: *Campylobacter spp.* are among the most important bacterial foodborne pathogens (Mead et al., 1999). One significant trend of antimicrobial resistance in this organism is emergence and spread of quinolone resistance. *Campylobacter jejuni*, the most important subspecies more commonly implicated in human campylobacteriosis, but less common in pigs than other food animals particularly poultry. However, *C. coli*, the second most common subspecies as a human pathogen is reported to be common among pigs. The objectives of this study were to determine the prevalence of *Campylobacter spp.* among market-age pigs, on-farm, at slaughter and post chilling and determine antimicrobial resistance frequency and patterns among groups of pigs raised ABF and conventionally.

Materials and Methods: A total of 60 farms were selected for entry into the project, 30 conventionally reared (antimicrobials included in the feed) and 30 antimicrobial-free (no antimicrobials included in the feed) during the growing phase. From three regions of the United States, 10 conventional and 10 antibiotic-free herds were recruited. Sampling consisted of 10 g of feces obtained from each of 30 pigs on-farm within 48 hours of slaughter. In addition, 10 carcass swabs were obtained at each of three points during the slaughter process; pre-evisceration, post-evisceration and post-chilling. Samples were enriched using Bolton broth at 42AC for 24 hours before plating on to Campy Cefex agar and incubated at 42AC for 48 hours under microaerophilic conditions. Presumptive colonies were confirmed using oxidase/catalase tests. Speciation was done using polymerase chain reaction (PCR) for *hipO*, *cadF*, and *ceuE* gene alleles. Antimicrobial susceptibility tests were done for 12 antimicrobials using Kirby-Bauer and epsilometric test (E-test) methods.

Results: Thus far, 14 farms, eight conventional and six antimicrobial-free from two regions have been completed. All 14 herds were positive for *Campylobacter*. As shown on Table 1, the overall on-farm prevalence of *Campylobacter* among ABF herds was 71% and 81% among conventional herd. In contrast, the prevalence among carcass swabs was higher among ABF herd than conventional herd with 60% and 30% respectively. Comparison of carcass swab samples at slaughter before and after chilling revealed that in all the carcass positive groups (13 of 14), there was significant reduction after chilling ($p < 0.05$). Comparing, the prevalence among post-chill samples between ABF and conventional herds, lower prevalence was detected among conventional herds than ABF, with 7% and 30% respectively (Table 1). Among 12 antimicrobials used to test susceptibility in the *Campylobacter* isolates, we detected resistance to seven antimicrobials. No resistance to ciprofloxacin detected. Comparing antimicrobial resistance for each of the antimicrobials to which resistance is detected between ABF and conventional herd frequency of resistance was significantly higher among isolates from conventional herd than ABF ($p < 0.05$) [Table 2]. As shown on this table, however, this finding was warranted when on-farm sample from the two production systems were compared. In contrast, sample from carcass swabs resulted in opposite trend. Frequency of resistance to five of the seven antimicrobials (except erythromycin and azithromycin) was higher among samples from carcass of ABF herds than conventional herds.

Table 1. Prevalence of *Campylobacter* among six ABF and eight conventional swineherds. Comparison of prevalence on-farm, pre-chill and post-chill is shown.

| Farm no. | ABF (% Prevalence) | | | Conventional | | |
|----------------|--------------------|-----------|------------|--------------|-----------|------------|
| | On-farm | Pre-chill | Post-chill | On-farm | Pre-chill | Post-chill |
| 1 | 72 | 60 | 0 | 93 | 0 | 0 |
| 2 | 52 | 90 | 50 | 85 | 45 | 0 |
| 3 | 76 | 25 | 0 | 97 | 28 | 5 |
| 4 | 86 | 50 | 0 | 94 | 55 | 0 |
| 5 | 72 | 50 | 0 | 93 | 23 | 0 |
| 6 | 100 | 100 | 100 | 80 | 40 | 5 |
| 7 | - | - | - | 43 | 20 | 10 |
| 8 | - | - | - | 68 | 30 | 0 |
| Average | 71 | 60 | 29 | 81 | 30 | 7 |

Table 2. Antimicrobial resistance among 50 isolates of *Campylobacter* isolated from six swine farms (3 ABF and 3 conventional herds).

| Antimicrobial | ABF (% resistance) | | | Conventional (% resistance) | | |
|------------------|--------------------|-----------|-------|-----------------------------|-----------|-------|
| | On-farm | Slaughter | Total | On-farm | Slaughter | Total |
| Ampicillin | 0 | 17 | 8 | 50 | 0 | 38 |
| Streptomycin | 0 | 25 | 12.5 | 55 | 0 | 42 |
| Sulfamethoxazole | 0 | 8 | 4 | 50 | 0 | 38 |
| Tetracycline | 8 | 17 | 12.5 | 75 | 0 | 58 |
| Erythromycin | 50 | 8 | 29 | 70 | 17 | 58 |
| Kanamycin | 0 | 8 | 4 | 20 | 0 | 15 |
| Azithromycin | 17 | 8 | 12.5 | 35 | 17 | 31 |

Discussion and conclusions: High prevalence of *Campylobacter coli* was detected in both ABF and conventional production systems. This is consistent with previous findings that *Campylobacter* is common among pigs. The interesting aspect, however, was that even though the prevalence was higher among conventionally-raised pigs on-farm, the opposite was true when carcass swabs were considered. As the pigs from ABF were slaughtered in an abattoir separate from the one used for conventional pigs, the result may be due to an abattoir effect. Significant reduction of prevalence was also noticed in all positive pig groups in post-chill samples. This was particularly true for the conventionally-raised groups. This can be explained by the presence of blast chiller system in the abattoir where the conventionally-raised pigs were slaughtered. Frequency of antimicrobial resistance among *Campylobacter* from the two production systems revealed that at the level of on-farm, frequency of resistance to the antimicrobials tested was lower among ABF pigs than conventionally-raised pigs. Even though this is not conclusive, as the study is still on-going, the findings so far have consistent trend. On the other hand the ABF pigs harbored higher frequency of resistant organisms from carcass swabs than conventional ones. This may be due to multiple factors including cross contamination at slaughter or sampling error.

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Trends in Salmonella shedding by U.S. market hogs, swine 2000.

O 08

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Summary: USDA's National Animal Health Monitoring System's (NAHMS) conducted a study of the health and management of swine as part of the Swine '95 study and Swine 2000 study. The studies were conducted in the top swine states in the U.S. A subset of participating farms allowed collection of fecal samples (50 samples per farm) from pens containing late finishers. The same methodology and laboratory was used in both studies so comparison of results provides a measure of change in

on-farm Salmonella status between 1995 and 2000. Samples were tested for Salmonella and questionnaires were used to obtain management data regarding feed management, environmental conditions, vaccination policies, and other factors. The percent of farms with at least one positive sample was 38% in 1995 and 34% in 2000. The percent of samples positive was 6% in 1995 and 2000. The percent of pens positive was 17% in 1995 and 16% in 2000. In contrast to trends in slaughter HACCP Salmonella samples, the similarity in on-farm Salmonella status suggests that on-farm Salmonella intervention has yet to be initiated on a wide scale by producers and that on-farm Salmonella status is not directly related to slaughter Salmonella status.

Keywords: NAHMS, Food Safety, swine, Salmonella, on-farm epidemiology

Introduction: Salmonellosis clearly is a perplexing problem that has challenged researchers and public health officials for decades and the complex ecology of the organism has hampered the identification of a simple means for effective control. Historical assumptions are that controlling Salmonella on farm will decrease risk of Salmonellosis further down the pork chain [Leistner et al., 1961]. This belief has been more widely accepted due to the intensified concern over microbiological hazards of food by a public wary of agriculture. The inability of traditional control methods (organoleptic inspection) to prevent transmission of food-borne pathogens, lead to the implementation of HACCP compliance monitoring by USDA [National Research Council, 1985]. The general assumption by many was that this would lead to greater pressure for on-farm intervention of Salmonella transmission.

Ensuring high-quality, safe, wholesome products is a cooperative effort throughout the pork industry. Since pork safety begins on the farm, producers and practitioners play a critical role in providing safe pork products for U.S. and international consumers [National Pork Board, 2002]. USDA's National Animal Health Monitoring System (NAHMS) seeks to provide the pork industry with objective, national information regarding food-borne pathogens associated with swine. Besides providing basic epidemiological on-farm description of these pathogens, the information collected from the previous two national swine studies allows for comparison of on-farm Salmonella results since implementation of HACCP. The purpose of this paper is to present descriptive Salmonella findings from the NAHMS Swine '95 and Swine 2000 studies to assess change in on-farm Salmonella status.

Methods: In 1995, NAHMS conducted a national swine study in the top 16 states. The design of this study has been presented elsewhere [Losinger et al., 1998]. Producers randomly selected by USDA's National Agriculture Statistical Service (NASS) and with at least 300 grower/finisher swine were eligible for the NAHMS Swine '95 Grower/Finisher study. Of the 418 participants, 152 were conveniently selected to contribute biological samples for isolation of Salmonella.

In 2000, NAHMS conducted a national swine study in the top 17 states. A complete description of the study design can be found in the Methodology section of Part I: Reference of Swine Health and Management in the United States, 2000 [USDA:APHIS, 2002]. Briefly, producers in the top 17 swine States with at least 100 total inventory on March 1, 2000 were randomly selected to participate in the study. Of the 895 participants, 127 were conveniently selected to contribute biological samples for isolation of Salmonella.

In both studies, the distribution of participants were allocated in proportion to the size of the pork industry in that State. A total of fifty fresh fecal samples were collected from the pen floors of late finishers (> 20 weeks of age), typically 5 samples from 10 pens. Each 25 gram sample was placed in whirl pack bags and shipped overnight to Russell Research Center in Athens, Georgia. Isolation methods have been described elsewhere [Davies et al., 2000].

Results: From the Swine '95 study, a total of 6655 samples were collected from 988 pens on 152 operations. At least one positive sample was found on 58 operations (38.2%) and a total of 414

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serotypes were isolated from 398 samples (6.0%). The 10 most frequent serotypes shed by finish hogs accounted for 85% of the isolates found in this study (see table 1).

From the Swine 2000 study, a total of 5509 samples were collected from 124 sites. At least one positive sample was found on 43 (34.7%) sites and a total of 363 (6.6%) samples were positive. The 10 most frequent serotypes accounted for 81.3% of the isolates shed by late finishers in this study(see table 1).

Table 1: Top 10 *Salmonella* serotypes isolated from late finisher pens in the NAHMS Swine '95 and Swine 2000 national studies.

| Swine '95 study | Swine 2000 study |
|--------------------|--------------------|
| 1. Derby | 1. Derby |
| 2. Agona | 2. Agona |
| 3. Typhimurium cop | 3. Typhimurium cop |
| 4. Brandenburg | 4. Heidelberg |
| 5. Mbandaka | 5. Brandenburg |
| 6. Typhimurium | 6. Typhimurium |
| 7. Heidelberg | 7. Worthington |
| 8. Anatum | 8. Anatum |
| 9. Enteritidis BA | 9. Infantis |
| 10. Worthington | 10. Tennessee |

Discussion: Two national studies were conducted five years apart, however the sampling methodology and laboratory procedures used were identical. In 1995, USDA also conducted HACCP Baseline studies for *Salmonella* contamination of carcasses. After five years, HACCP compliance monitoring has shown a decrease in contamination levels of market hog carcasses. From 8.7% in 1995 Baseline to 3.8% in 2001 for all plants sizes[USDA:FSIS, 2003]. However, based on the Swine 2000 study, the *Salmonella* status of late finishers on-farm is unchanged since 1995.

There are a few notable implications to this observation. First, that pressure for on-farm *Salmonella* intervention has not materialized to any great extent as evidenced by the identical levels of *Salmonella* in late finishers. One explanation could be the widespread adoption of ineffectual interventions, however fewer than 10% of sites applied any *Salmonella* intervention strategies in 2000 [USDA:APHIS, 2002]. A second implication is the revelation of a disconnect between *Salmonella* contamination at slaughter and *Salmonella* infection at the farm. These two points are certainly related as few producers will be willing to implement costly intervention strategies without assurances that they impact not only *Salmonella* levels on farm, but slaughter plant levels of *Salmonella* contamination.

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O 09

Risk factors for *Yersinia Enterocolitica* on U.S. swine farms in 2000

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Summary: The NAHMS Swine 2000 study was conducted in the top 17 swine producing states to determine the animal prevalence of *Yersinia enterocolitica*. Producers were randomly selected and their participation solicited for various portions of the study. Questionnaires were used to obtain management data regarding feed management, environmental conditions, vaccination policies, and other factors. Fecal samples were collected from 129 sites, 50 samples per site from 10 pens with late finishers. Tonsil swabs were taken from 12 late finishing pigs per site on 115 sites. A total of 2664 fecal samples and 1180 tonsil swabs were screened for *ail* gene by PCR. Positive samples were placed on culture (ITC broth and CIN agar) and results were merged with questionnaire data. Half of the sites (58/115) were classified as positive. A logistic regression model was constructed for each variable which passed the screening cut-off p value of <0.25 controlling for region. Final factors associated with positive farm status for *Yersinia enterocolitica* (*ail*+) included inclusion of bone meal in finisher diet, any grower/finisher deaths due to scours, and vaccination for E coli.

Keywords: NAHMS, Food Safety, swine, *Yersinia enterocolitica*, on-farm epidemiology

Introduction: In 1995 the Animal Production Technical Analysis Group subcommittee on Risk and Health Impact ranked *Yersinia enterocolitica* as one of the top six priority foodborne pathogens based on acute and chronic health effects in the United States [Buntain, 1995]. In a keynote address to the American Association of Swine Veterinarians, Peter Davies recognized *Yersinia* as the Achilles' heel of the U.S. pork industry with respect to food-borne pathogens [Davies, 1999]. *Yersiniosis* is characterized by mild to severe diarrhea streaked with mucous or blood, fever, abdominal cramps and pain, nausea. In 3-15% of cases it mimics appendicitis. It primarily affects children under 7 years of age, more often boys than girls [Kapperud, 2002].

Yersinia enterocolitica has been found in dogs, cats, rabbits, cattle, horses, sheep, goats, deer, elk and many types of birds and rodents [Mollaret et al., 1979]. Outbreaks in the past have been attributed to contaminated chocolate milk, water, tofu, and shellfish. More recent studies have discovered the bacterium in the oral cavity, feces, and intestinal contents of healthy pigs at slaughter. The similarity of these isolates to those found in humans has implicated swine as the principal source for human contamination [Schiemann, 1980; Doyle et al., 1981].

The purpose of this study was to estimate the animal-level prevalence of pathogenic (*ail*) *Yersinia enterocolitica* based on PCR analysis of tonsil swabs from 12 finishers on 150 farms. Associations with farm health and management data would be analyzed to form hypothesis on potential interventions and/or implications of *Yersinia enterocolitica* infection in swine.

Materials and Methods: The NAHMS Swine 2000 study was conducted in the top 17 swine producing

states of the US. Producers were randomly selected and their participation solicited for various portions of the study. Response rates and a profile of participants can be found in the back of the Swine 2000 Part III Reference report [USDA:APHIS, 2002]. The subset of participants that were invited to participate in the foodborne pathogen sampling component of the study were allocated across the 17 states in rough proportion to their contribution to the US pork industry (a composite of number of producers and total hog inventory). Questionnaires were completed on each of three face-to-face interviews with the first visit being conducted June-July 2000. On-farm sampling by federal and state field veterinarians was conducted from August through October 2000 and December 2000 to March 2001. Tonsil swabs were shipped overnight to USDA:ARS-NADC in Ames, IA (Wesley). Feces were shipped overnight to USDA:ARS lab in Athens, GA for processing. A subset of fecal samples was sent to USDA:ARS in Ames, IA (Wesley) and USDA:ARS in Wyndmoor, PA (Bhaduri) on alternating weeks. All samples were enriched in ITC media for 2 days (at room temp). Wesley then extracted DNA for confirmation of *ail* gene using a fluorogenic 5' nuclease PCR assay [Boyapalle et al., 2001]. After enrichment, Bhaduri streaked selective media (CIN agar) and one typical *Yersinia* "bull's eye" colony was selected for *ail* confirmation via PCR. A logistic regression model was constructed from each variable that was significantly associated with *Yersinia* status controlling for region. Variables were placed in a full model if the Wald chi-square statistic had a p value < 0.25. A reduced model was generated based on the four variables which contributed most to the significance of the full model, i.e. those with a Wald chi-square value greater than 2 (and therefore a p value < 0.1).

Results: Tonsil swabs were taken from 1180 late finishing pigs (9.3 swabs per site) on 127 sites. There were 119 (10.1%) swabs positive for pathogenic *Yersinia enterocolitica*. Pen floor fecal samples were collected from up to 10 pens with late finishers (20 weeks of age or older). Of the 2664 samples collected, 340 (12.8%) were positive. Overall, 58 of the 127 sites (45.7%) had at least one positive tonsil swab or fecal sample.

The prevalence of positive sites was lower in Northern and Southern tier states (33-37%) compared to central 'cornbelt' states (57.1%). Sites sampled in the fall were less likely to be positive (40.7%) than those sampled in the winter (59.3%).

The likelihood ratio testing the global null hypothesis for the full model was 0.006 indicating that some or all of the variables contributed to a model that was significantly better than a model with all coefficients equal to 0. Table 1 lists the significant variables which were incorporated into the full model. Variables tested but found insignificant include herd size, outdoor access, waste management, AI/AO pig flow, livestock trucks coming on farm, and cats on premise. The reduced model presented in Table 2 as the final logistic regression model for estimating risk factors associated with sites positive for *Yersinia enterocolitica* is not significantly different than the full model.

Table 1: Full logistic regression model of variables significantly associated with *Yersinia enterocolitica aii* positive sites from the NAHMS Swine 2000 study.

| Variables passing screening | Percent positive | | P value | |
|--------------------------------|---------------------|-----------------|-------------|------|
| Deworm regularly | Yes 58.3 | No 44.6 | 0.15 | |
| Antibiotics in feed | Yes 54.3 | No 31.6 | 0.07 | |
| Vaccination for <i>E. coli</i> | Yes 61.0 | No 44.4 | 0.09 | |
| Percent deaths due to Scours | 0 % 45.7 | 1-100% 66.7 | 0.07 | |
| Atrophic Rhinitis - GF | Yes 63.2 | No 48.3 | 0.24 | |
| Meat/bone meal in GF diet | Yes 66.7 | No 47.1 | 0.10 | |
| Animal/Veg. fat in GF diet | Yes 44.0 | No 56.9 | 0.18 | |
| Buildings restrict rats/mice | None 59.6 | Some 48.3 | All 38.5 | 0.19 |
| Feral pigs in county | Yes 70.0 | No 49.5 | 0.21 | |
| Salmonella shedding in GF | Yes 54.9 | No 42.1 | 0.20 | |
| Region | Non-central 36.1 | Central 57.1 | 0.04 | |

ORAL PRESENTATIONS

Table 2: Final reduced logistic regression model of variables significantly associated with *Yersinia enterocolitica* all positive sites from the NAHMS Swine 2000 study.

| Variables in final model | P value | Odds Ratio | 95% CI |
|---|---------|------------|------------|
| Region (Central states) Non-central states | 0.013 | 0.3 | 0.1 – 0.8 |
| Vaccination for <i>E. coli</i> (No) Yes | 0.023 | 3.0 | 1.2 – 7.5 |
| Percent deaths due to Scours (0%) 1-100% | 0.022 | 3.5 | 1.2 – 10.5 |
| Meat/bone meal in GF diet (No) Yes | 0.017 | 4.1 | 1.3 – 13.3 |

Discussion: Even though pork has been implicated as a common source for Yersiniosis in people as evidenced by the common isolation of human strains in pork products, there are no published reports estimating prevalence of pathogenic *Yersinia* in swine on farms. This paper provides an initial look at the epidemiology of pathogenic strains of *Yersinia* on swine production sites in the US. The percent sites positive is similar to estimates obtained in slaughter-based studies [Funk et al., 1998] and on-farm studies in Chile and Italy. Higher isolation rates were obtained in cooler months which is also consistent with previous studies [Funk et al., 1998].

Despite previous studies suggesting the tonsil is most reliable source for isolation of *Yersinia* from swine, in this study a greater proportion of fecal samples (12.8%) were positive than tonsil swabs (10.1%). Slaughter based studies however typically harvest tonsil tissue which may provide superior isolation rates to tonsil swabs. Nevertheless, testing of fecal samples may provide an acceptable method of determining *Yersinia* status for on-farm epidemiological studies.

Sites in the central states were more likely to be positive than sites in the southern states (33%) or northern states (37%) which may reflect environmental influences on agent survival and/or other production management differences not evaluated in this study. The association with *E. coli* vaccine use and finisher deaths attributed to scours may indicate opportunistic colonization of *Yersinia* afforded under these conditions. As *Yersinia enterocolitica* is sensitive to heat, the use of meat and bone meal (MBM) is not likely to be explained as a contaminated source for infection of swine. Given the strength of the association found in this study, however, the connection with *Yersinia* infection in swine on-farm requires further study.

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The Sero-prevalence of *Salmonella* spp. in Finishing Swine in Iowa

O 10

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Keywords: food safety, surveillance, meat-juice, zoonosis, market swine

Summary: This study represents the first attempt to classify Iowa production sites for *Salmonella* spp. sero-prevalence. The data suggest that the Iowa herds are similar in their distribution with respect to sero-prevalence of salmonella as Danish herds. Ignoring herd size, 91.2% of surveyed herds were negative or level 1, 8.2% were level 2 herds, and 1.6% level 3. These results are similar to previous Danish studies (Alban et al., 2002, Mousing et al., 1997). The current data suggests that larger herds tend to have a higher sero-prevalence than smaller units; however, formal analysis has yet to be conducted to determine the direct association between herd size and salmonella sero-prevalence. Studies by Carstensen et al. (1998) suggested that herd size was statistically associated, albeit weakly, with *Salmonella* sero-prevalence, but the authors concluded the relationship was probably not biologically significant.

Introduction: The study objective was to estimate the *Salmonella* serologic prevalence of production sites in Iowa.

Methods and materials: The study population was 7150 Iowa producers who submitted at least one lot (lot sizes – 20- 180 head) per month - March to February 2002. Four diaphragm samples were collected from each lot as part of an Aujeszky's Disease surveillance program. A total of 1131 farms met the criteria. *Sample analysis:* Meat juice samples were tested by the IDEXX Swine Salmonella test kit[®]. The cut-off positive value was designated by the manufacturer. *Statistical analysis:* For the individual animal the prevalence and 95% CI were calculated using Proc Surveymeans (SAS 8.1[®]). For lots and farms the sampling probability was unknown, therefore Proc Means (SAS 8.1[®]) was used. Farms were classified based on their estimated annual production and sero-prevalence. Farms with < 5000 annual production were classified: negative, level 1 (0% - < 25% sero-positive), level 2 (25 - 50%) and level 3 (> 50%). Farms with > 5000 annual production were classified: negative, level 1 (0% - < 10% sero-positive), level 2 (10 - 33%) and level 3 (> 33%).

Results: From a total of 28,465 samples collected, 26,325 were used in this analysis. The remaining samples were from herds that did not produce sufficient numbers within the sampling time period. Of those selected, 24,403 samples tested negative, 1915 positive and seven samples could not be tested. These 26,325 samples were collected from 6521 harvest lots. In 1229 lots at least one animal tested positive with 1915/5200 positive. Their average lot prevalence was 38.8%. At least one positive animal was submitted from 506 farms. Farms with < 500 head annual production were classified: negative- 232/319; Level 1 - 57/319; Level 2 - 25/319; and Level 3 - 5/319. Farms with between 500 - 5000 head were classified: negative - 371/784; Level 1 - 340/784; Level 2 - 61/784; and Level 3 - 12/784. Farms with > 5000 head the classifications were: negative - 7/28; Level 1 - 14/28; and Level 2 - 7/28. Figure 1 gives the percentages of levels within herd sizes. Figure 2 provides information about the estimated sero-prevalence ignoring herd size. Figure 3 gives the frequency distribution histograms for each herd size.

Discussion: This study represents the first attempt to classify Iowa production sites for *Salmonella* spp. sero-prevalence. The data suggest that the Iowa herds are similar in their distribution with respect to sero-prevalence of salmonella as Danish herds. Ignoring herd size, 89 % of herds were negative or level 1, 9.5 % were level 2 herds and 1.5 % were level 3. This is very similar to the results reported by Mousing et al., (1997): 93 % of Danish herds were negative or level 1, 3.9 % where level 2 and 2.3 % where level 3. The data however do suggest that larger herds may tend to have a higher sero-prevalence (Figures 1 and 3). This interpretation however is limited by the fact that few herds are large in this dataset - only 28, but again this follows a similar pattern observed in Danish studies in 1997 (Mousing et al., 1997). Studies by Carstensen et al. (1998) suggested that herd size was statistically associated with *Salmonella* sero-prevalence in Danish herds but the authors concluded the relationship was probably not biologically significant. This analysis has yet to be conducted on Iowa herds to determine if these herds differ from Danish herds with respect to the ecology of salmonella.

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Figure 1: The cumulative percentage of levels of Salmonella sero-prevalence in Iowa herds

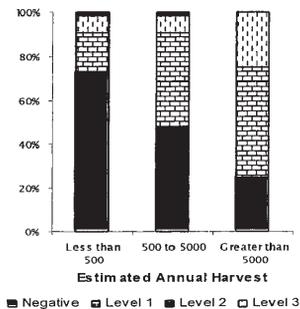


Figure 2: Frequency distribution of sero-prevalence of 6652 lots in Iowa

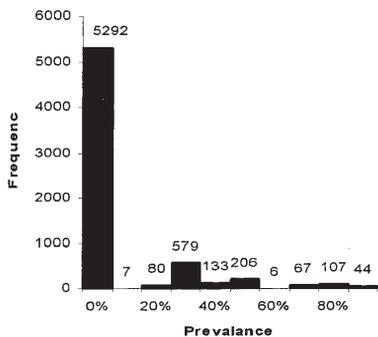
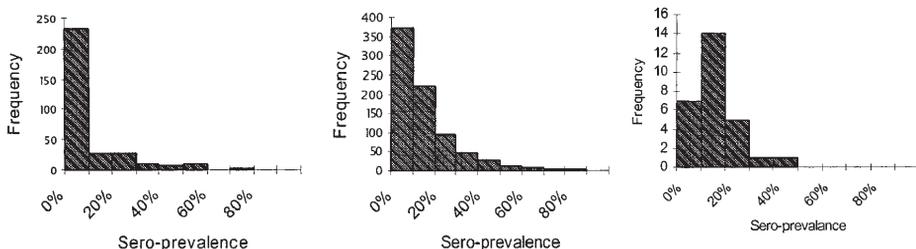


Figure 3: Frequency distribution of sero-prevalence by annual herd slaughter size (left annual harvest < 500; middle annual harvest, 500 to 5000; right, annual harvest > 5000)



Risk Factors for Swine Infection with *Toxoplasma gondii*

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Summary: The objective of this study was to evaluate the association between the seroprevalence of *Toxoplasma gondii* antibodies in swine in commercial pork production systems in Iowa, USA and the source of water (surface water vs. well water vs. rural processed water) and method of water delivery (surface or trough vs. nipple vs. cup). Also the study evaluated the association between *T. gondii* seroprevalence and other selected potential farm variables.

In this study the following on-farm risk factors had a statistically significant association with swine being seropositive for *T. gondii*:

1. In adult swine those that drank well water were more likely to be seropositive than those that drank pipe-delivered rural water;
2. In grow/finish swine those that had water delivered through surface water or trough water were more likely to be seropositive;
3. Non-confinement housing resulted in a higher likelihood of being seropositive in all groups of swine studied; and
4. Failure to clean facilities between groups of animals resulted in a higher likelihood of being seropositive in all groups of swine studied.

Keywords: Food Safety, *Toxoplasma gondii*, Pork, Toxoplasmosis, Preharvest Pork Safety.

Introduction: *Toxoplasma gondii*, a protozoan parasite capable of infecting all mammals and birds, is the causative agent of one of the most common parasitic global zoonoses (Tenter et al., 2000). Even though *T. gondii* infections in humans are often asymptomatic, the organism still causes an estimated 112,500 cases of foodborne illness in the U.S. annually and, together with two other pathogens, accounts for 75% of the foodborne-illness deaths attributable to known agents (Mead et al., 1999). When *T. gondii* is considered as a foodborne pathogen, pork is often singled out as the most likely carrier food of the organism. This perception stems from past studies which indicate that swine have a high seroprevalence of antibodies against *T. gondii* relative to other food-producing animals (Davies 1999). The results of the 2000 USDA National Animal Health Monitoring System's National Swine Survey, the most recent comprehensive study of the U.S. pork industry, found an infection rate of 0.8% for finisher pigs and an infection rate of 5.78% for sows (Bush 2002). This infection rate is significantly lower than in previous studies of the pork industry. With this knowledge and with the progress that is being made on the Trichinae Certification Program, the U.S. pork industry is considering developing an on-farm auditing system to document good production practices that will decrease swine exposure to *T. gondii*. Initial steps in the development of this system involve the discovery of all on-farm risk factors for swine infection with the *T. gondii*.

Materials and Methods: Fifteen swine serum samples from each of 414 pork production sites throughout Iowa were obtained through the pseudorabies surveillance program. Samples were tested for the presence of antibodies against *T. gondii* using the modified agglutination test (MAT). The MAT uses formalin-fixed tachyzoites as antigen. Antibody titers ≥ 32 were considered positive, i.e., that the pig had been infected with *T. gondii* at some time during its life. Information about the source and method of water delivery, as well as other relevant production information, was obtained via

telephone interview from the veterinarian who had submitted the serum samples for the site.

Results: A total of 6210 animals were tested in the study, with 422 (6.8%) testing seropositive. Swine from all levels of production were included in this study, but a majority of the swine tested were finishers and sows. Of the 414 Iowa pork production sites in the study, 97 (23.4%) had at least one seropositive animal. The following on-farm risk factors had a statistically significant association with swine being seropositive for *T. gondii*:

1. In adult swine: well water vs. pipe-delivered rural water ($p = 0.0012$);
2. In grow/finish swine: surface or trough water vs. all other delivery methods ($p = 0.0026$);
3. Non-confinement housing (grow/finish $p = 0.0063$; adult $p = 0.0049$);
4. Failure to clean facilities between groups of animals (grow/finish $p = 0.0516$; adult $p = 0.0026$).

Discussion: If *T. gondii* as a food safety issue is to be addressed through the implementation of a preharvest risk mitigation system, all of the on-farm risk factors for swine infection with the organism must be clearly delineated so that risk reduction and/or elimination strategies can be devised. Previous studies have identified most of the on-farm risk factors for swine infection with *T. gondii* (Lubroth et al., 1983, Smith et al., 1992, Assadi-Rad et al., 1995, Weigel et al., 1995, & Dubey et al., 1986). However, the source of water for the herd was not evaluated as a risk factor for *T. gondii* infection of swine. Other research has demonstrated that ingestion of water contaminated with *T. gondii* oocysts is an increasingly common route of infection in humans (Kourenti et al., 2003, Bahia-Oliveira et al., 2003, Hunter et al., 2001, Bowie et al., 1997, & Benenson et al., 1982). The importance of water transmission in swine is unknown. Oocysts can survive in water for long periods of time under a variety of conditions (Dubey 1998), but they have not been recovered from water sources on swine farms (Dubey et al., 1995).

Conclusions: The relative importance of a variety of on-farm risk factors needs to be understood to reduce *T. gondii* infection in swine. This study demonstrates that a variety of risk factors, including the source and method of water delivery, need to be addressed to control *T. gondii* infection on pork production sites. This information is needed before a preharvest risk mitigation system for pork production facilities can be developed.

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Individual effect of the steps preceding slaughtering on *Salmonella* contamination of pigs.

O 12

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Summary: The influence of the different steps preceding pig slaughtering (waiting in the herd, transport and lairage) was studied regarding deep (organs) and surface (carcass) *Salmonella* contamination, by mixing SPF with contaminated pigs at the different steps. For a lairage of 2 hours and a transport of 1 hour, the caecal contamination concerned the conventional pigs and the long time mixed groups (more than lairage time). The isolated strains were from herd origin according to serotyping. After the slaughtering process, it was not possible to differentiate carcasses contamination rate for the conventional batch from those of the control group pigs (transport and lairage in *Salmonella*-free conditions). This study showed that without efficient control measures during slaughtering, implementation of control measures in the herd would be inefficient regarding carcass contamination rate.

Keywords: lairage, mixing groups, carcasses and organs contamination.

Introduction: The risk of surface contamination of the pig carcasses is associated with the level of contamination of the digestive tract of the animal (Berends et al., 1997). According to this, many efforts focused on the definition of the circumstances associated with a shedder status for pigs from a given breeding at the end of the growing period. An important difference appeared between the status as they can be described in the breeding and the one obtained after an evaluation after slaughter (Hurd et al., 2002). To obtain benefits of the application of on herd measures taken to control *Salmonella*, we have to better understand the individual role of the different steps preceding the slaughtering. This was the aim of our study and we proposed to check the following steps for *Salmonella* contamination : waiting at the herd, transport, lairage and slaughtering process.

Materials and methods: Pigs: 60 conventional pigs (Conv) issued from a farm where *Salmonella* were found in environmental swab and faecal matter (Table1). 48 SPF pigs (aged of 136 days for an average weight of 102 kg) produced in experimental equipments regularly controlled for the absence

of *Salmonella*. **Experimental design** : the first 12 SPF pigs group (group A) arrived at 15:00 in the contaminated herd. They were mixed with the Conv batch when they entered the waiting room of the herd (waiting for the transport truck). The next morning the second group of SPF pigs (group B) arrived in a transport truck. They hadn't any contact with the herd except they have been added to the previously mixed group of pigs A and Conv in the truck. This new batch has been transported to an hour's drive away slaughterhouse. In parallel two other 12 SPF groups left the experimental room to the slaughterhouse at a one hour of way trip. The group D waited in the truck and constituted the control group. The groups A+ B + Conv and C entered simultaneously in the same lairage. After the 2 hours lairage time, all the pigs entered the slaughtering process. **Bacteriological evaluation** :*Salmonella* search included BPW pre-enrichment, two selective enrichments (in Semi Solid Rappaport Vassiliadis medium and Mueller Kauffman tetrathionate broth). Enrichments were respectively streaked on Rambach agar or XLT4 medium. Five typical colonies were picked on each agar plate. A total of 10 isolates per positive sample were biochemically checked for *Salmonella* and serotyped according to the Kauffmann-White scheme. Quantification: the mini-MSRV method was used to quantify *Salmonella* in positive caecal samples. This method is based on the Most Probable Number method and used the selectivity of the MSRV medium in miniaturised conditions (Fravallo et al., 2002). **Sample** : environmental sampling consisted in large swabbing of floor surfaces of the pen where pigs have waited. The contamination was observed before and after the passing of the pigs. Samples realized on pigs after slaughtering consisted in : caecal content (25g), mesenteric lymph nodes, stomach content (25g) just after evisceration. After the chilling of carcasses, amygdalae were individually excised and the half of the carcasses was individually and extensively swabbed.

Results: The selected herd was considered positive as it allowed the detection of *Salmonella* in 50% of the environment swabs and in faecal matter of the studied batch. All of the 31 isolates presented a particular serotype SI 4,12:i:-. (Table 1).

Table 1: Serological results for *Salmonella* per isolated serotype

| Sample | Detection | Number of isolates | Serotype |
|--------------------------------------|-------------|--------------------|--------------|
| Environment swabs (wall of the pens) | 2/8 | 3 | SI 4,12:i:-. |
| Pools of faecal mater | 2/4 | 3 | SI 4,12:i:-. |
| Swabs of the quay | 4/4 | 25 | SI 4,12:i:-. |
| Total | 8/16 | 31 | |

The pigs A, C and D were transported in SPF conditions, as the surfaces of the trucks didn't show *Salmonella* contamination (Table 2). The herd waiting quay was contaminated before (4/4 positives samples) and after (3/4 positives samples) the passing of the batch (pigs A and Conv). All isolates, except one Typhimurium, belonged to SI 4,12:i:- serotype. The pigs entered in a imperfectly decontaminated truck. Only one sample out of four showed *Salmonella* contamination with no less than 3 different serotypes. After the transport of the batch (conv +A+B), the serotype SI 4,12:i:- appeared in the composition of the contaminated samples. The lairage pen was positive (3/3samples) for *Salmonella* detection and allowed the identification of 2 serotypes in the same proportion Typhimurium and Derby. After the batch passing, the SI 4,12:i:- serotype was found in the pen swab.

Table 2: Environment conditions during the experimentation

| Sample | Detection | Nb of isolates | Serotype (number) |
|---|-----------|----------------|--|
| SPF transport of the group A to the Conv herd | | | |
| Swabs before | 0/2 | - | |
| Swabs after | 0/2 | - | |
| Waiting quay at the herd | | | |
| Swabs before | 4/4 | 25 | SI 4,12:i:- (25) |
| Swabs after | 3/4 | 20 | SI 4,12:i:-(19) Typhimurium (1) |
| Transport to the slaughterhouse for groups A, B and Conv | | | |
| Swabs before | 1/4 | 10 | Senftenberg (5); Typhimurium (4); Derby (1) |
| Swabs after | 3/3 | 24 | Derby (13); Typhimurium (7); SI 4,12:i:-(4) |
| SPF transport of the group C and D to the slaughterhouse | | | |
| Swabs before | 0/2 | - | |
| Swabs after | 0/2 | - | |
| Waiting quay at the slaughter house | | | |
| Swabs before | 3/3 | 25 | Typhimurium (15);Derby (10) |
| Swabs after | 5/5 | 37 | Derby (25); SI 4,12:i:- (3); Typhimurium (9) |

Stomach samples didn't show *Salmonella* except for 1 sample in Conv pigs and in one pig of the B group. The serotyping showed that this contamination was recently acquired. Amygdales were sporadically contaminated as shown by the group D (only one Derby serotyped positive sample) (Table3). The sampling occurred after the chilling.

Table 3: Contamination after slaughtering. (Typ. : Typhimurium, Der : Derby SI : SI 4,12:i:-)

| Sample | Group A Serotypes (nb of isolates) | Group B Serotypes (nb of isolates) | Group C Serotypes (nb of isolates) | Group D Serotypes (nb of isolates) | Conv Serotypes (nb of isolates) |
|-------------|--|--|--|--|---------------------------------------|
| Caecum | 3/12 SI (19) Typ (1) | 1/12 SI(1) | 0/12 | 0/12 | 3/12 SI(15); Typ (9) |
| Lymph nodes | 1/12 SI(1) | 0/12 | 1/12 Typ (10) | 0/12 | 5/12 Typ (28); SI(22) |
| Amygdales | 0/12 | 0/10 | 0/11 | 1/9 Der (10) | 1/12 Der (10) |
| Carcasses | 6/12 Typ (22) Der (33) | 3/11 Der (30) | 7/12 Der (52); Typ (18) | 5/11 Typ (26); Der (24) | 9/12 Der (49); Typ (41) |
| Stomach | 0/12 | 1/12 Der (6) | 0/12 | 0/12 | 1/12 Der (5) |

Caecal contamination was not shown for the groups C and D. For the pigs B and C, acquisition of *Salmonella* was found for respectively 1/12 and 3/12. The proportion of caecal contamination was the same for the Group A and the conventional pigs. The serotype was SI 4,12:i:-, confirming a herd origine for the *Salmonella* present in the neocontaminated pigs. A quantitative evaluation of the positive caecal content was realized and showed for the neocontaminated (group A) as well as for the conventional pigs, that the *Salmonella* concentration in the caecal content was below the MPN 0.67/gram IC95% 0.11-5.4.

The proportion of positive carcasses appeared to be independent of the considered pig group. It was not possible to differentiate Conv pigs from those originally SPF. Moreover the serotype of the strains obtained from the carcasses swabs were Typhimurium and Derby. Despite the number of tested isolates (305) the serotype SI 4,12:i:- was not identified on the positive carcasses.

Discussion: The identification of a SI 4,12:i:- serotype in the herd was a great opportunity to describe the contamination of the SPF pigs with the herd serotype. This serotype could be considered as

Typhimurium lacking the secondary flagella phase. As one isolate of Typhimurium was described in the herd after the waiting and without further discriminating typing, the serotype Typhimurium could not be attributed neither for a herd origine nor for a slaughterhouse one. However Derby was clearly acquired after leaving the herd (potentially during the transport but more presumably during the lairage). Despite the succession of the commonly applied stress on pigs, the quantification of the caecal contents of conventional and neo-contaminated pigs allowed to confirm that the excretion is not massive in the described conditions. In this study we could not compare the obtained percentage of positive carcasses with previously published data as the entire surfaces were swabbed. However, we could observed that the proportion of positive carcasses was not defined by the status of the group before the slaughtering : Conv group, group A and group D could not be differentiated. Moreover the proportion of the different serotypes *i.e.* the majority of Derby and the absence of SI 4,12:i:-, allowed to associate the carcass contamination to the last steps of the slaughtering process. The contamination of the caecum appeared to be a function of the duration of the contact time with non-SPF conditions. In this study, the 2 hours waiting in contaminated conditions seems to be insufficient to contaminate SPF pigs (see group C pigs). The transport with contaminated pigs allow to obtain contaminated pigs (at least 1/12) but with the herd serotype. Under the studied conditions and at the carcass contamination level, the slaughtering process hid the initial difference of pig status regarding *Salmonella* contamination.

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Epidemiology of salmonellosis in fattening units of Catalonia (Spain): A bacteriological survey

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Summary: A bacteriological survey was conducted on a representative sample of swine farms of Catalonia. In each farm, fecal samples from finishing pigs were collected and a detailed questionnaire was filled. Results of bacteriological examinations and data gathered in the questionnaires were analysed by using Epi-Info 2002. Variables with p-values <0.20 were included in a logistic regression analysis. A total of 113 fattening units were examined, corresponding to 2187 samples of which 46 were positive (2.10 %), accounting for 14 different serotypes. The percentage of farms in which *Salmonella* carriers were detected corresponded to 20.35 %. Within positive farms, 10.0 % of samples were positive. Logistic analysis showed that the presence of other species of livestock and the number

of pigs in the farm were significant factors of risk. Poultry represented the species that most likely introduce salmonellosis in pig farms (Odds ratio = 3.71). Also, a past history of clinical salmonellosis was significant ($p < 0.05$). Our results showed that about 2 % of the pigs and between a fifth and a quarter of farms have active *Salmonella* excretors. This high value should be taken into account when implementing future plans for *Salmonella* control in swine.

Keywords: Salmonella, Swine, Excretors, Farms, Livestock.

Introduction: In the last years, food safety has become one of the top priorities for most governments in Western countries. Classically, it has been considered that pig played a secondary role in the transmission of this infection for humans. However, some serious outbreaks of human salmonellosis demonstrated that pigs or pig meat products could be traced back as the source of disease in some cases. Any strategy to control salmonellosis in pigs necessarily needs a deep understanding of the epidemiology of this infection in swine. The aim of the present study was to determine the prevalence of fattening units of Catalonia having active *Salmonella* excretors and to figure out what risk factors can contribute to the presence of this infection in fattening units.

Materials and methods: A bacteriological survey was conducted on a representative sample of swine farms of Catalonia. The initial hypothesis was that at least 15 % of the farms would have active *Salmonella* carriers in fattening units. Considering that the total census of pig farms is 12,512, the total fattening units to be examined was calculated to be 108 (± 7.5 % precision, confidence level 95 %). If a farm was positive it was considered that, at least, 15 % of the animals would be active carriers. This implied to sample 19 animals per unit. Fecal samples were collected and inoculated in Rappaport-Vassiliadis broth. Incubations were done at 42 °C and transfers to XLT4 agar were done after 24 and 48 hours of incubation. Suspicious colonies were further identified by routine biochemical tests and serotyping. For each farm a detailed questionnaire was filled including 84 questions about health, production, facilities, medications and husbandry practices of the surveyed farms. Results of bacteriological examinations and data gathered in the questionnaires were analysed by using Epi-Info 2002. Variables yielding p -values < 0.20 were further included in a logistic regression analysis.

Results and discussion: At the end of the study, 113 fattening units were examined, corresponding to 2187 samples of which 46 were positive (2.10 %), accounting for 14 different serotypes. The percentage of farms in which *Salmonella* carriers were detected corresponded to 20.35 % (95 % confidence intervals: 13.59 % - 29.18 %). Within positive farms, 10.0 % (7.49 % - 13.20 %) of samples were positive. Epidemiologic analysis of the results showed that the presence of other species of livestock and the number of pigs in the farm were significant factors of risk. Poultry represented the species that most likely introduce salmonellosis in pig farms (Odds ratio = 3.71 (1.11-12.39), $p = 0.032$). Also, a past history of clinical salmonellosis was significant (Table 1).

Table 1. Significant variables in the logistic regression model

| Variable | Odds Ratio (95 % Confidence interval) | p-value |
|---|--|---------|
| Other livestock in farm | 5.82 (1.55-21.83) | 0.009 |
| >1600 fattening pigs in farm | 4.76 (1.21-20.0) | 0.020 |
| Past history of clinical salmonellosis | 4.03 (0.90-18.05) | 0.004 |
| MLE of the model: 81.67 % , $p = 0.004$ | | |

Our results showed that about 2 % of the pigs and between a fifth and a quarter of farms have active *Salmonella* excretors. This is in agreement with previous reports (Barber *et al.*, 2002). This high value should be taken into account when implementing future plans for *Salmonella* control in swine. On the other hand, the results of the epidemiological analysis showed that mixing of different livestock species

is an important risk factor. This fact has been reported previously (Funk *et al.*, 2001) and confirms that this practice should be avoided if salmonellosis is to be controlled. On the other hand, large fattening units were more prone to have positive animals than small ones. One possible explanation is that these large units usually receive animals from different source farms, increasing thus the chance to introduce infected pigs. Taken together, these results indicate that salmonellosis is widespread in swine farms of Catalonia (Spain) and that implementation of control measures is urgently needed.

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O 14

Epidemiology of salmonellosis in sow units of Catalonia (Spain)

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Summary: A bacteriological study aimed to the detection of sows excreting *Salmonella* was carried out in 74 sow units. Individual faecal samples were collected and a detailed questionnaire was filled in each farm and the data gathered were used in a bivariate logistic regression analysis to determine risk factors for *Salmonella* positivity. In 18 farms (24.32 %) *Salmonella* carriers were detected. Of the total 1480 samples analysed, 50 were positive (3.38 %), accounting for 11 different serotypes. Within positive farms, 13.8 % of sows were positive. Epidemiologic analysis of the results showed three main risk factors: to have open-flushed drainage of slurry, rodent control, and the number of sows in the unit. Our results showed that, in infected farms, the proportion of sows actively excreting *Salmonella* was considerably high (3.38 %). This fact, and the risk factors detected should be taken into account when designing effective plans for the control of salmonellosis in sows.

Keywords: Salmonella, Carrier, Risk factors, Catalonia, Swine

Introduction: Salmonella infection is a recognised problem in pig-meat production. The greatest emphasis in reducing *S. enterica* contamination has focused on finishing swine. A study demonstrated that sows are a potential source for *S. enterica* (McKean *et al.*, 2001). However, detailed scientific results are fewer in this age group.

The aim of the present study was to determine the prevalence of sow units of Catalonia having active *Salmonella* excretors and to figure out what risk factors can contribute to the presence of this infection in sow units.

Materials and methods: A bacteriological survey was conducted on a representative sample of swine farms of Catalonia. The initial hypothesis was that at least 20 % of the farms would have active *Salmonella* carriers in sow units. Considering that the total census of pig farms with sow units is 5742, the total number of farms to be examined was 61 (± 10 % precision, confidence level 95 %). If a farm was positive it was considered that, at least, 15 % of the animals would be active carriers. This level

implied to sample 19 animals per unit. Individual fecal samples were collected and inoculated in Rappaport-Vassiliadis broth. Incubations were done at 42 °C and transfers to XLT4 agar were done after 24 and 48 hours of incubation. Suspicious colonies were further identified by routine biochemical tests and later serotyped. For each farm a detailed questionnaire was filled. This questionnaire included 84 questions about health, production, facilities, medications and husbandry practices of the surveyed farms. Results of bacteriological examinations and data gathered in the questionnaires were analysed by using Epi-Info 2002. Variables yielding p-values < 0.20 were further included in a logistic regression analysis.

Results and discussion: A total number of 74 sow units were finally examined. In 18 of them (24.32 %, 95 % confidence interval 15.42 % - 35.93 %) *Salmonella* carriers were detected. Of the total 1480 samples analyzed, 50 were positive corresponding to an overall individual prevalence of 3.38 % (2.54 % - 4.46 %). Within positive farms, 13.8 % of sows were positive. Eleven different serotypes were isolated. The statistical analysis of the results showed three main risk factors: to have open-flushed drainage of sewage, rodent control, and the number of sows in the unit (Table 1). However, the logistic regression model only explained 33 % of the cases, indicating other important risk factors that could not be identified.

| Variable | Odds Ratio (95 % Confidence interval) | p-value |
|-------------------------------------|--|---------|
| Open-flushed slurry drainage | 34.48 (1.22-100.0) | 0.03 |
| Rodent control implemented | 0.054 (0.003-0.87) | 0.03 |
| >250 sows in farm | 9.26 (1.15-74.62) | 0.03 |
| MLE of the model: 33.78 %, p= 0.003 | | |

Table 1. Significant variables in the logistic regression model

Several studies have shown that sows have an important role in the epidemiology of salmonella in swine (Kranker *et al.* 2001; Letelier *et al.* 1999). In our study about one fourth of the examined units had active *Salmonella* carriers indicating a wide extension of the infection. Most important, the proportion of active excretors in infected farms was considerably high, a fact that implies an increased risk of transmission to offspring. Most often, the positive status in sows is related to the type of feed used (Kranker *et al.*, 2001). However, in our case we were not able to identify any of the feeding-related variables (pelleted feed, etc.) as a risk factor. In contrast, rodents and the type of drainage of slurry were detected as significant risk factors. Rodents are known to be carriers of *Salmonella*. Barber *et al.* (2002), detected 8 % of rodents as active excretors of *Salmonella* in swine farms, having thus a possible role as reservoirs. Regarding the number of sows in the farm, this might be an indirect indicator of the number of replacement gilts introduced in the herd. On the other hand, slurry is also a source of contamination for birds, rodents and also can contaminate boots, etc. As a conclusion, a considerable proportion of the investigated sows were found to be actively infected. This fact, and the risk factors detected should be taken into account when designing effective plans for the control of salmonellosis in sows.

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The prevalence and risk factors of porcine cysticercosis in Zambia

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Summary: The objectives of these studies were to determine the prevalence and importance of porcine cysticercosis in Zambia. Lingual examination of live pigs and visual inspection of their carcass as well as blood sampling for measuring circulating parasite antigen by enzyme-linked-immunosorbent assay (Ag-ELISA) were used as parameters to determine infection. During the field surveys a questionnaire was also administered to every household whose pigs were examined to obtain information on husbandry practices and study risk factors associated with the infection. A total of 1416 free-range pigs were examined at slaughter slabs in Lusaka and 950 pigs have been examined in field surveys in Southern and Eastern provinces. Four hundred and seventy three farmers were interviewed using a questionnaire. The abattoir surveys indicate prevalence of 11% by tongue examination and 54.8% by Ag-ELISA. The field surveys showed 15.8% positive by lingual examination and 28% were found to be positive by Ag-ELISA. It is clear from the 473 farmers interviewed that most risk factors for transmission and sustainability of pork tapeworm are available. These include free ranging, lacked latrines, pork consumption, pigs home slaughtered without inspection and eating *cysticerc*-infected pork. These studies indicate that the pork tapeworm is a serious agricultural problem and poses a substantial public health hazard to the population especially in rural Zambia.

Keywords: Ag-ELISA, Neurocysticercosis, *Taeniosis*, Tapeworm.

Introduction: Data collected during the last decade show that *T. solium* cysticercosis in pigs and man is more widely distributed in sub-Saharan Africa than previously assumed (Geerts *et al.* 2002, Phiri *et al.* 2001, Phiri *et al.* 2002, Phiri *et al.* 2003). The cysticerci of *T. solium* may lodge in the brain causing cerebral cysticercosis (neurocysticercosis), a very serious zoonosis causing headache, epileptic seizures, epilepsy, mental disturbance and death. The *T. solium* taeniosis/cysticercosis complex is associated with poor sanitation and hygiene, poor pig husbandry and poor meat inspection and control. However, precise data on the prevalence and importance of this zoonotic disease in Zambia are scarce.

Materials and Methods: The studies were done in Eastern and Southern Provinces but a limited number of pigs also came from Western and central Provinces. The surveys started in 1999. The pigs slaughtered were between 6 months and several years and both sexes were included. At slaughter the presence of cysticerci was assessed by examining cyst predilection sites in the carcass including the masseter muscles, hind leg muscles, tongue, heart and psoas muscles.

In the both field and slaughter slab surveys pigs were examined for the presence of cysticerci by tongue palpation (Gonzales *et al.*, 1990) and blood was collected by puncture of the cranial vena cava or the jugular vein into plain tubes. Serum was separated and dispensed into aliquots and stored at -20°C until analysis. The Ag-ELISA was performed as described by Dorny *et al.* (2000) with a few modifications. Information on the environmental, demographic and risk factors associated with transmission of *T. solium* within the surveyed communities were recorded by way of questionnaires.

Results: Of the 1416 pigs examined at the slaughter slab 156 pigs (11%) were found to be positive by lingual examination. 21.3% (302) were found positive at meat inspection meaning that 145 pigs (48%) with cysticercosis at meat inspection were not detected by lingual examination. And 54.8% of them were positive by Ag-ELISA. Thus far 950 pigs have been examined in field surveys with 15.8% positive by lingual examination and 28% positive by Ag-ELISA. Of the 473 farmers interviewed, 74.9% kept pigs under free-range conditions, 53.4% lacked latrines, 92.4% consumed pork, 96.6% slaughtered pigs at home without inspection, 32.1% admitted eating *cysticercus*-infected pork, 61% were ignorant about cysticercosis while 42.7% obtained drinking water from rivers and shallow wells. The studies showed that 40.4% households were positive for porcine cysticercosis having at least one positive pig.

Discussion: It was shown in these studies that tongue palpation is a very specific method to demonstrate cysticercosis in pigs, but that it has a sensitivity not exceeding 70% this is in agreement with Gonzales et al., 1990. Therefore, the value of tongue palpation in community-based studies does not reflect the extent of the prevalence.

There are a number of factors in the surveyed villages contributing to optimal conditions for transmission of this cestode include the free-roaming of pigs, lack of latrines, the absence of meat inspection and the lack of awareness of the local population, of the risks involved in eating meat with *T. solium* cysts. Although statistical analysis failed to show associations between infection in pigs and various epidemiological factors, the study population was fairly homogeneous in terms of most of these factors and the pigs appear to be equally and permanently at risk of coming into contact with the parasites. The high prevalence of cysticercosis in pigs strongly suggests that many people are carriers of the pig tapeworm and consequently contaminate their direct environment with eggs containing oncospheres that are infective for man. In regions where cysticercosis in pigs is common, human cysticercosis prevalence is usually high. Results from other endemic areas in Africa (Newell *et al.*, 1997) have indicated a strong relationship between cysticercosis and epilepsy. Therefore, there is an urgent need to collect baseline data on human cysticercosis in Zambia for a better understanding of the local epidemiology and the transmission risks.

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Serological research of *Salmonella* on Belgian pig farms

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Summary: Risk factors for Salmonellosis in pigs were investigated in a cross-sectional study on 144 Belgian farrow-to-finish herds belonging to one slaughterhouse co-operation. Herd data were collected using a questionnaire. The blood samples were serologically analyzed. Variables significantly related to the *Salmonella* prevalence in the univariate analyses were subsequently analysed in a multivariate model. Furthermore, the clustering of *Salmonella* infection within the herd, section and pen was studied. The average within-herd seroprevalence was: 73.4% when using OD 10%. In the multivariate analyses the structure of the feed seems to be the most important factor of the model with five factors. Feeding pigs meal instead of granulated or crumb is a protecting factor for *Salmonella*. Other risk factors in the multivariate model are natural ventilation, less than 3 days emptiness after wet cleansing, not dry cleansing of sows' pen before wet cleansing and the absence of dogs in the pig houses.

Keywords: within-herd seroprevalence, risk factors, s/p ratio

Introduction: For reasons of food safety and because of economic pressure, risk factor studies are required to have a scientific basis to initiate a control programme for *Salmonella* in pig herds in Belgium. The aim of the present study was to determine risk factors for the seroprevalence of *Salmonella* in Belgian slaughter pigs. This study was based on serological analysis on blood samples and differs from other risk factor studies in the level of analysis, i.e. at pig level. (Lo Fo Wong., 2001). Possible clustering of *Salmonella* infections between pigs from the same herd, section and pen was also investigated and corrected for in the risk factor analysis study.

Materials and Methods: From each of the 144 farrow-to-finish herds, 50 randomly selected pigs from an average delivery of 95 pigs were blood sampled at slaughter. The blood samples were serologically analyzed with an indirect ELISA (HerdChek) (Idexx Laboratories, Inc.). A questionnaire, consisting of 2 major parts, was used to collect the herd data. A general part concerned all pigs in the herd, a specific part concerned the slaughter pigs to be sampled. In both parts, following topics were included: housing and ventilation, management, hygiene and biosecurity and production parameters. The specific part additionally pertained to feeding, disease control and transport to the slaughterhouse. All study pigs were individually identified before transport to the slaughterhouse.

For the determination of the risk factors a Linear Mixed Model was used, PROC MIXED in SAS® release 8.02. This method was used with the S/P ratio as the depending variable on the pig level for the following reasons: - there are 3 possible cut-off values that could be used; - for each pig the farm, section and the pen are known, so that correcting for clustering is possible, and possible getting additional information about the spread of *Salmonella* (Lo Fo Wong., 2001); - analyses on pig level and with a continue depending variable have more power than analysis on herd level with a dichotomised variable. In a first step, each of the factors obtained from the questionnaire were separately introduced in the model to assess whether any of these factors was univariately associated with the S/P ratio. Categorical and continuous variables significantly related to the S/P ratio ($p < 0.05$) were analysed jointly in a multivariate mixed model with herd, section and pen as random effect.

Results: In 142 (98.6%) herds, at least 1 sample was positive when using OD 10% as cut-off, 97.6% (OD 20%) and 91% (OD 40%). The average within-herd seroprevalence was: 73.4% (range 0 – 100%) when using OD 10% as cut-off, 51.8% (range 0 – 100%) (OD 20%), 30.1% (range 0 – 98%) (OD 40%). There was substantial and significant variation between the herds, with the variance being estimated as 0.023 (mean =0.39; s.e. =0.24). Categorical and continuous variables were studied by univariate analysis, of which 73 were significantly associated with *Salmonella* prevalence ($p < 0.05$). The most important variables were selected for the multiple analyses based on the p-value, the estimate and the biological sense. The variables were introduced in the multivariate model. Following factors are significantly associated with the S/P ratio on pig level: less than 3 days emptiness after wet cleansing ($p = 0.0026$; estimate = 0.082), natural ventilation in the pig stables ($p = 0.0164$; estimate = 0.081), the absence of dogs in the pig stables ($p = 0.0042$; estimate = 0.078) and not dry cleansing in the sows stable before wet cleansing ($p = 0.0225$; estimate = 0.076). Besides these four factors the structure of the feed seems to be the most important ($p = 0.0002$; estimate = 0.114). Feeding pigs meal instead of granulated or crumb is a protecting factor. These five factors incorporated in the multivariate model could reduce the variance between herds to 0.013 or 43.5 % could be explained by this factors. An Interclass Correlation Coefficient (ICC) of 0.36 was calculated for samples within herds, ICC = 0.54 for samples within sections and ICC = 0.68 for samples within pens.

Discussion: This study was based on serological results namely the S/P ratio of the sample. Using this S/P ratio instead of the dichotomous variable negative or positive has a few advantages in this study. For each pig farm, section and pen are known, so that correction for clustering was possible on all these levels, making a selection for a cut-off value unnecessary. From the multivariate model the most important factor seems the structure of the feed. Feeding pigs meal instead of granulated feed or crumb is a protecting factor for *Salmonella*. Jorgensen et al. (1999) found that pigs that received meal have the largest population of lactic acid bacteria in the stomach. The difference between meal and granulated feed can be explained by differences in the coarseness (Kjeldsen and Dahl, 1999). Beside the feed also hygienic measures and ventilation seems to be important. We found a high ICC on herd, section and pen level, which means that results of samples within a herd; section and pen have a tendency to be the same (van der Wolf P.J., 2000).

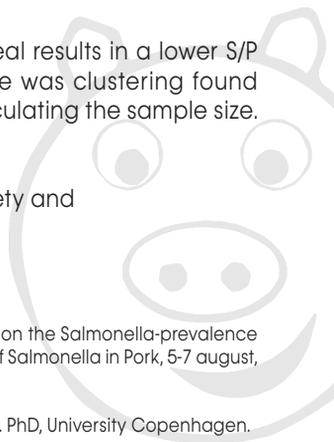
Conclusions: The statistical method can be used in this study. Feeding meal results in a lower S/P ratio value for *Salmonella* compared to granulated feed or crumb. There was clustering found between pigs from the same herd, section and pen this can be used in calculating the sample size.

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RISK FACTORS FOR THE PREVALENCE OF *SALMONELLA* IN BELGIAN SLAUGHTER PIGS

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Summary: Risk factors for Salmonellosis in pigs were investigated in a cross-sectional study on 62 Belgian farrow-to-finish herds belonging to one slaughterhouse co-operation. Herd data were collected using a questionnaire. The outcome variable, the percentage of positive animals per slaughterhouse delivery, was determined by qualitative *Salmonella* isolation from the mesenteric lymph nodes. Variables significantly related to the *Salmonella* prevalence in the univariate analyses were subsequently analysed in a multivariate model. Furthermore, the clustering of *Salmonella* infection within the pen was studied. The median percentage of positive samples per delivery was 64.5%. In the multivariate model, only type of floor significantly influenced the prevalence independently ($p < 0.05$) with a fully slatted floor leading to the lowest *Salmonella* prevalence. Clustering between pigs from the same pen could not be demonstrated. The risk factors investigated here could only explain a small amount of the variability between herds.

Keywords: bacteriology, mesenteric lymph nodes, between-herd variability, clustering

Introduction: For reasons of food safety and economic pressure, risk factor studies are required to have a scientific basis to initiate a control programme for *Salmonella* in pig herds in Belgium. The aim of the present study was to determine risk factors for the prevalence of *Salmonella* carriers in Belgian slaughter pigs. This study was based on bacteriological isolation in mesenteric lymph nodes and differs from other risk factor studies in origin of sample (van der Wolf et al., 1999; Lo Fo Wong et al., 2002). Because the animals were individually identified, possible clustering of *Salmonella* infections between pigs from the same pen was also investigated.

Materials and Methods: From each of the 62 farrow-to-finish herds, 30 randomly selected pigs from an average delivery of 95 pigs were sampled at slaughter. A questionnaire, consisting of 2 major parts, was used to collect the herd data. A general part concerned all pigs in the herd, a specific part concerned the slaughter pigs to be sampled. In both parts, following topics were included: housing and ventilation, management, hygiene and biosecurity and production parameters. The specific part additionally pertained to feeding, disease control and transport to the slaughterhouse. All study pigs were individually identified before transport to the slaughterhouse. After evisceration, the intestines were numbered. Mesenteric lymph nodes were collected from every study pig and transported to the laboratory for qualitative *Salmonella* isolation, using standard procedures.

A generalised mixed model with binomially distributed error term was fitted to the percentage of positive animals in a slaughterhouse delivery. In a first step, each of the factors obtained from the questionnaire were separately introduced in the model to assess whether any of these factors was univariate associated with the risk of *Salmonella* infection. Factors significantly related to the *Salmonella* prevalence ($p < 0.05$) were analysed jointly in a multivariate generalised mixed model with herd as random effect. Correlation between pigs from the same pen was studied in a generalised mixed model with binomially distributed error term and introducing pen as random effect and herd as fixed effect (PROC NLMIXED SAS 8.02).

Results: In 57 (91.9%) herds, at least 1 sample was positive. The overall prevalence was 65.6% (range 0 – 100%). There was substantial and significant variation between the herds, with the variance being estimated as 7.74. Eighteen categorical variables were studied by univariate analysis, of which only 6 were significantly associated with *Salmonella* prevalence ($p < 0.05$): region ($p = 0.014$), purchase of gilts ($p = 0.011$), presence of the cat in the stables ($p = 0.011$), type of floor in the finishing unit ($p = 0.005$), ventilation type in the nursery unit ($p = 0.043$) and slaughterhouse ($p = 0.001$). The first 4 variables were introduced in the multivariate model. Slaughterhouse was excluded for reasons of multicollinearity with region. Ventilation type in the nursery unit was excluded because of 7 missing values. Only type of floor influenced the *Salmonella* prevalence ($p < 0.05$) with a fully slatted floor associated with the lowest *Salmonella* prevalence. These factors incorporated in the multivariate model could only reduce the variance between herds to 4.51. The variance of the pen effect was not significantly different from zero (0.11 ± 0.16), so no clustering between pigs from the same pen could be demonstrated.

Discussion: This study was based on *Salmonella* isolation in mesenteric lymph nodes collected at the slaughterhouse and differed from other risk factor studies in origin sample. The determination of the prevalence in mesenteric lymph nodes gives a good indication of the carrier state of *Salmonella* infected animals, which are important for contamination of carcasses (Swanenburg et al., 2001). Although *Salmonella* infections during transport cannot be excluded, it is not considered important in the present study, because of the short transport and lairage times in Belgium. The variability for within-herd prevalence between herds was found high. The factors incorporated in the multivariate model could only partly explain the variability between herds, other potential risk indicators should be investigated in further research. Fully slatted floors seemed to be associated with the lowest *Salmonella* prevalence, in comparison with partly slatted or solid floors. In pens with fully slatted floors, less contact with contaminated faeces is possible and re-infection cycles are less likely to occur (Davies et al., 1997).

In all study herds, nose to nose contact between pigs from neighbourhood pens was possible. When a *Salmonella* infection enters the house, it can easily spread through all pens what can explain that no clustering was found.

Conclusions: A fully slatted floor results in a lower *Salmonella* prevalence compared to partly slatted or solid floors. Other risk factors need to be investigated. No clustering between pigs from the same pen was found.

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YERSINIA PREVALENCE IN ANTIBIOTIC FREE AND CONVENTIONALLY REARED SWINE

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Summary: These preliminary results compare the on-farm prevalence and frequency of carcass contamination with *Yersinia enterocolitica* (YE) for pigs reared under conventional and antibiotic free production systems. At the time of submission, results are available for 6 herds. In 5 of 6 herds, we were able to isolate YE from at least one pig on-farm. The overall individual pig prevalence was 13.3% on conventional farms 59.3% on ABF farms. YE was isolated from 2 of 191 carcass swabs. Although the preliminary nature of these results limits interpretation, it supports previous results that indicate swine serve as a reservoir for YE.

Introduction: *Yersinia enterocolitica* is a food-borne pathogen causing an estimated 96,000 Americans to become ill each year (Mead, 1999). Swine are considered the primary reservoir of pathogenic YE. In a previous survey of US market pigs (Funk et al., 1998), over 28% of herds have been estimated to be positive for pathogenic (*ail* gene-harboring) YE. As part of multi-state study to determine the prevalence, antimicrobial resistance and genotypic diversity of three major foodborne pathogens in swine: *Salmonella*, *Campylobacter* and *Yersinia*, the goal of the present study was to compare the prevalence of YE in market swine reared under conventional and antibiotic-free (ABF) production.

Methods: A total of 60 farms were selected for entry into the project, 30 conventionally reared (antibiotics included in the feed) and 30 antibiotic free (no antibiotics included in the feed) during the growing phase. The farms were equivalently distributed in 3 regions of the United States. Within each region, 10 conventional and 10 antibiotic free herds were recruited. A questionnaire was administered in person to record farm antibiotic use and management practices. A schematic of sampling protocol is shown in Table 1. On-farm sampling consisted of 10 g of feces obtained from each of 30 pigs (or the entire marketing if less than 30) on farm that were destined for harvest within 48 hours. Sampling of carcasses at the slaughter plant was conducted in the following manner. Carcasses were sampled at each of 3 points during the slaughter process; pre-evisceration, post-evisceration and post-chilling. Of the 30 pigs sampled on farm, 10 carcasses were sampled at pre-evisceration, 10 at post-evisceration and 10 post-chilling. One swab was conducted per carcass at pre-evisceration and post-evisceration, and 2 swabs were obtained from carcasses post-chill. The swabbing technique at pre- and post evisceration consisted of a bung-to-jowl swipe along the evisceration incision with a 7.62 X 12.70 cm sterile cellulose sponge (Solar-cult cellulose sponge, Solar Biologicals, Ogdensburg, NY, USA) dampened with 20ml of buffered peptone water. At post-chilling, the same protocol was conducted on one side of the carcass. On the other side of the same carcass, a modification of the USDA sampling method for HACCP monitoring (Anonymous, 1996) was conducted. Briefly, the same locations were swabbed (belly, bung, jowl), but with the 7.62 X 12.7 cm sponge using a template with 3 times the area of the standard protocol. Swabs were placed in Whirl-pak bags (Nasco, Ft. Atkinson, WI, USA) and transported to the lab on ice. Swabs were divided into 3 equal portions using sterile technique, with each 1/3 swab portion placed in a sterile Whirl-pak. One sub-sample of the swab was cultured for YE. The other swabs were forwarded to collaborating investigators for isolation of other foodborne pathogens. Feces and swabs were cultured utilizing cold enrichment in

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phosphate buffered saline for 21 days followed by plating on cefsulodin-Irgasan-novobiocin agar (*Yersinia* Selective Agar, CIN, Becton Dickinson and Company, Franklin Lakes, NJ, USA). Colonies with morphology typical of *YE* were then biochemically screened by subculture onto Kligler's Iron Agar and urea broth (Becton Dickinson and Company). Isolates biochemically typical of *YE* were then further characterized by presence or absence of the *ail* gene by PCR and by serotyping (O1, O3, O5, O8, O9, *Yersinia* antisera, Denka Seiken, Tokyo, Japan). Antimicrobial resistance was determined against 17 antimicrobials (Sensitire, Trek Diagnostics, Cleveland, Ohio, USA).

Results: At the time of submission, 6 farms (4 conventional and 2 ABF) have complete results through presumptive identification of *YE* by biochemical testing (Table 2). Five herds (3 conventional and 2 antibiotic free) have had at least one *YE* isolate from fecal samples. One herd had 2 positive swab samples. The prevalence of pigs positive for *YE* as determined by fecal culture on farm was 13.3% on conventional farms and 59.3% on ABF farms. Serotype distribution, PCR analysis for detection of the presence or absence of the *ail* gene and antimicrobial resistance are pending. The preliminary nature of this data precludes us from forming conclusions from these results. These data do support previous investigations that suggest swine can serve as a reservoir of *YE*.

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Table 1. Schematic of target sampling scheme for each participating pig herd.

| | | Slaughter Plant Sample Scheme | | | | |
|------------|----------|-------------------------------|------------------|-------------------|--------------|---------------|
| | | On Farm | Pre-evisceration | Post-evisceration | Post-Chill | |
| | | | bung to jowl | bung to jowl | bung to jowl | Modified USDA |
| Pigs 1-30 | 30 fecal | | | | | |
| Pigs 1-10 | | 10 swabs | | | | |
| Pigs 11-20 | | | 10 swabs | | | |
| Pigs 21-30 | | | | | 10 swabs | 10 swabs |

Table 2. Prevalence of *YE* in herds with biochemical results at time of submission.

| Farm | Conventional Antibiotic Free* | or | Fecal | Pre- evisceration | Post- evisceration | Post -chill | |
|-------|----------------------------------|----|--------|----------------------|-----------------------|-------------|--------------|
| | | | | | | Bung/Jowl | Mod. USDA |
| 1 | C | | 0/28 | 0/9 | 0/5 | ND | 0/12 |
| 2 | C | | 9/35 | 0/10 | 0/10 | ND | 0/10 |
| 3 | ABF | | 15/22 | 0/10 | 0/10 | 0/10 | 0/10 |
| 4 | C | | 5/22 | 0/10 | 1/10 | 0/10 | 1/10 |
| 5 | C | | 1/28 | 0/10 | 0/10 | 0/10 | 0/10 |
| 6 | ABF | | 1/5 | 0/5 | 0/5 | ND | 0/5 |
| Total | | | 31/140 | 0/54 | 1/50 | 0/30 | 1/57 |

*C=conventional, ABF=antibiotic free

Tuberculous lesions in pigs in the Czech Republic in the years 1990-1999

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Summary: In the Czech Republic, bovine tuberculosis in cattle was controlled in 1968. The last outbreak was diagnosed in cattle and domestic pigs in 1995. During the veterinary hygiene inspection of pigs slaughtered in slaughterhouses, however, tuberculous lesions were still being found above all in the head and intestinal lymph nodes. In the decade monitored a total of 45 873 318 pigs were slaughtered and examined according to veterinary hygiene standards. Apart from 1991, when results of tuberculous findings were not obtained, tuberculous lesions were found in 134 088 (0.32%) of the 41 458 565 pigs examined in the remaining nine years. During a detailed analysis of the pathological anatomical examination of 190 940 pigs slaughtered in one district, tuberculous lesions in lymph nodes were found in 4 107 (2.2%) pigs: mesenteric (65.3% pigs), submandibular (18.6% pigs), inguinal (0.1% pigs) and simultaneously intestinal and head lymph nodes (15.9% pigs). Miliary tuberculosis was found only in the parenchymatous organs of four (0.1%) pigs. The following financial losses resulted: 6% for confiscating the head, intestines and stomach, and 22 to 24% for assessing meat as conditionally edible after processing, i.e. intended only for heat-processed products. Mycobacteria were isolated from 7 246 (41.8%) pigs through the cultivation of tissue samples from 17 326 pigs. *Mycobacterium bovis* was detected in only five (0.07%) animals which originated from the last outbreak of bovine tuberculosis in cattle in the Czech Republic in 1995. *M. avium* complex (MAC) isolates came from 6 870 (94.8%) animals: 55.7% *M. a. avium* isolates were mainly of serotypes 2 and 3 and genotype IS901+ and IS1245+ and 39.2% *M. a. hominissuis* isolates were mainly of serotypes 4, 8 and 9 and genotype IS901- and IS1245+. Conditionally pathogenic mycobacteria (*M. chelonae*, *M. terrae*, *M. phlei* and *M. fortuitum*) were isolated from 371 (5.1%) pigs. In the whole period monitored, two marked increases in the findings of tuberculous lesions were recorded: In the mid-1990s as a result of using deep bedding with wood shavings and at the end of the 1990s as a result of supplementing the pigs' feed with peat.

Keywords: meat inspection, mycobacteriosis, pig carcasses, risk assessment, PCR

Introduction: In the Czech Republic, bovine tuberculosis was controlled in cattle and other domestic animals in 1968 (Polak, 1969). The last infection caused by *Mycobacterium bovis* in domestic pigs was diagnosed in 1995 (Pavlík *et al.*, 2002). During the veterinary hygiene inspection of pigs slaughtered in slaughterhouses, however, tuberculous lesions were still being found. Pig breeders of animals affected in this way suffered major economic losses (Pavlík *et al.*, 2003). From pig lymph nodes with tuberculous lesions were isolated not only *Mycobacterium avium* (MAC) complex members but less commonly *Rhodococcus equi* (Dvorska *et al.*, 1999). The objectives of this work were the assessment for tuberculosis lesions of all pigs slaughtered in the Czech Republic during the years 1990-199 and the analysis of tissue cultures from pigs.

Materials and Methods: Between 1990 and 1999, when a total of 45 873 318 pigs were slaughtered in slaughterhouses in the Czech Republic, biological material was collected from 17 326 (0.04%) pigs for laboratory examination. Tissue samples were supplied to the laboratory immediately after collection, or were frozen and delivered in this condition no more than one month later for laboratory examination described previously (Fischer *et al.*, 2000). Mycobacterial isolates were identified using biochemical

methods (Wayne and Kubica, 1986), the Accu-Probe Probe (Accu-Probe Inc., San Diego, Calif.) system, PCR for the detection of IS901 (Kunze *et al.*, 1992) and IS1245 (Guerrero *et al.*, 1995). MAC isolates were also identified by serotyping (Wolinsky and Schaefer, 1973) and biological experiment on pullets (Pavlik *et al.*, 2000). *R. equi* isolates were identified according described methodology (Dvorska *et al.*, 1999).

Results: During veterinary hygiene inspection, tuberculous lesions were found in 134 088 (0.32%) of the 41 458 565 pigs slaughtered. The highest incidence of tuberculous lesions identified in pigs was found in 1990 (0.45%) and 1996 (0.43%). From a total of 17 326 pigs examined, mycobacteria were isolated from 7 246 (41.8%) animals. *M. bovis* was detected in 1995 only in the tissues of five pigs reared together with cattle in the last outbreak of bovine tuberculosis in cattle in the Czech Republic. A total of 6 870 (94.8%) MAC isolates were detected: 55.7% *M. a. avium* isolates and 39.2% *M. a. hominissuis* isolates. Statistically highly significant ($p < 0.01$) MAC isolates were identified more frequently from mesenteric lymph nodes (48.6%) compared to their identification from head lymph nodes (34.8%). The identification of MAC isolates from inguinal lymph nodes (4.3%) was statistically highly significantly lower ($p < 0.01$) than the identification of MAC isolates from head or mesenteric lymph nodes. A total of 371 (5.1%) isolates were identified as conditionally pathogenic species (*M. chelonae*, *M. terrae*, *M. phlei* and *M. fortuitum*). Over the years 1996 and 1999, from the lymph nodes of 1 745 pigs, *R. equi* was solely isolated from 154 (8.8%) pigs and in mixed infection with mycobacteria in a further 49 (2.8%) pigs. Statistically highly significant *R. equi* ($p < 0.01$) was isolated more frequently from head lymph nodes (21.0%) compared to intestinal lymph nodes (0.9%). Out of 190 940 pigs slaughtered, tuberculous lesions were found in 4 107 (2.2%) pigs, of which tuberculous lesions were found in the mesenteric lymph nodes of 65.3% pigs, submandibular lymph nodes of 18.6% pigs and inguinal lymph nodes of 0.1% pigs. Tuberculous lesions were found simultaneously in the intestinal and head lymph nodes of 15.9% animals. Miliary tuberculosis in parenchymatous organs (liver, spleen and kidneys) was only diagnosed in four (0.1%) pigs. Generalised chronic tuberculosis was not found in any pig.

Discussion: The first sharp increase of tuberculous lesions occurred in the mid-1990s, when enzymatically split sawdust began to be more commonly used in pig rearing as deep bedding (Bartl *et al.*, 1997). Subsequently, therefore, because of the increased discovery of tuberculous lesions in pigs, most pig-breeders gradually gave up this technology with deep bedding using sawdust (Pavlik *et al.*, 2003). The second increase in incidence of tuberculous lesions in the lymph nodes of pigs occurred at the end of the 1990s, when peat began to be used as an addition to feed for piglets for 2 up to 4 weeks after birth. The conditionally pathogenic mycobacteria isolated from the organs of pigs with tuberculous lesions were identical to the species isolated from peat (Matlova *et al.*, 2003).

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O 20 Genetic relatedness of *Salmonella enterica* isolates from pens and swine at slaughter

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Keywords: food safety, zoonosis, abattoir, lairage, PFGE

Summary: The study aimed to determine if *Salmonella enterica* isolates from the floor of pre-slaughter holding pens were genetically related to isolates found in swine, held in those pens, post slaughter. Pulsed-field gel electrophoresis (PFGE) typing was used to determine genetic relatedness. On seven occasions, 100% homologous PFGE patterns were found, i.e. the pen and pig isolates were identical. This suggested that pen to pig transfer of *Salmonella enterica* occurred. Isolates from PFGE patterns associated with pig to pen transfers were more likely to occur in the *S. Anatum*, *S. Heidelberg* and *S. Typhimurium* serotypes. The ability of an isolate from a pen to rapidly infect animals housed in the pen may vary within serotype based on factors described by the PFGE pattern. This may explain why some *S. enterica* serotypes are prevalent in swine but not in pork products or humans.

Introduction: In a study examining samples collected from the holding pen prior to pigs entry and then from the gastrointestinal tract of swine after slaughter Rostagno et al.(2003) observed that 26% of swine *S. enterica* isolates were the same serotype as found in the pens. This finding, and others, suggest that the holding pen in abattoirs is a significant source of *Salmonella enterica* in swine entering the slaughter floor (Hurd et al. 2001;McKean et al. 2001;Rostagno et al. 2001). However, similarities in serotype are only suggestive that isolates are related. Therefore, a study was conducted to determine the genetic relatedness, as determined by PFGE, of isolates of the same serovar found in the pre-slaughter holding pen and the pigs at slaughter.

Materials and methods: *Observational study design:* Three replicates were conducted at two high capacity abattoirs. Each replicate included four groups (150 pigs/group). For each group, the holding pens were sampled (6 pooled samples per pen, consisting of floor swabs, and residual liquids and feces) prior to the pigs entering. After holding and slaughter, cecal contents and ileocecal lymph nodes were collected from 30 pigs randomly selected from each group. *Sample processing:* All samples were processed by bacteriological methods for the isolation and identification of *Salmonella enterica*. After culturing, isolates were serotyped at the USDA National Veterinary Service Laboratory (NVSL), in Ames, Iowa. *PFGE profile determination:* For the isolates of interest, those with pen-pig serotype matches identified previously by Rostagno et al.(2001), the genetic profiles were determined using PFGE. *Data analyses:* **Step 1.** A number was assigned to each unique PFGE pattern. **Step 2.** For each run, pen and serotype the data were examined for the presence of 100% homologous PFGE patterns in the pigs and the pen. When a unique PFGE pattern occurred in a pig and the pen this was designated as a pig-pen PFGE pattern match (Table 1). **Step 3.** When a pig-pen PFGE pattern match was found, that pattern number was assigned the attribute "PFGE match". This designated that based on matching PFGE patterns it appeared that the isolates had transferred from the pen to the pig (Table 1). **Step 4.** Contingency tables were used to compare the prevalence of isolates from "PFGE match" patterns across the serotypes using Fishers exact test. The null hypothesis was that the proportion of isolates from "PFGE match" patterns was equal across all serotypes.

Results: The number of PFGE patterns identified with 90% and 100% homology, by serotype, are shown in Table 2. The 100% PFGE pattern pig-pen matches occurred on 7 occasions in 6 clusters. These clusters contained 50 isolates (bottom row of Table 3). The 90% PFGE pattern pig-pen matches occurred on 15 occasions, in 4 clusters. These four clusters contained 192 isolates. The proportion of isolates from "PFGE match" patterns was not equal across all serotypes ($p < 0.001$). A greater proportion of *S. Anatum* ($n=27/70$, 38%), *S. Heidelberg* ($n=4/12$, 33%) and *S. Typhimurium* ($n=11$, 24%) isolates belonged to 100% "PFGE match" patterns i.e. with pig-pen PFGE matches (Table 3), compared to the *S. Derby* and *S. Infantis* for which only 10% (8/82) and no (0/11) isolates respectively belonged to PFGE patterns associated with pig-pen transfers.

Discussion and conclusion: Serotyping and PFGE patterns are methods of categorizing bacteria isolates. We found an association between having a PFGE pattern associated with a pig-pen match and serotype. That is, some serotypes, *S. Anatum*, *S. Heidelberg* and *S. Typhimurium*, were more likely to contain isolates which possessed PFGE patterns associated with pig-pen matches. The ability of an isolate from the pen to rapidly infect animals housed in the pen may vary within serotype based on factors described by the PFGE pattern. This may explain why some *Salmonella enterica* serotypes are prevalent in swine but not in pork products or humans.

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Table 1: Example of a method of determination of a pig-pen "PFGE match" and assigning that phenotypic characteristics to a PFGE pattern. This example is for replicate one, pen one, *S. anatum*.

| 100% pattern no. | PFGE | pig | pen | Phenotype assigned to cluster |
|---------------------|------|-----|-----|----------------------------------|
| 45 | | 2 | | No Match |
| 52 | | 1 | 1 | PGFE Match |
| 69 | | | 1 | No Match |

Table 2: The count of 100% and 90% PFGE patterns and the number of isolates (in brackets) found within each serotype

| | S.Anatum | S.Derby | S.Heidelberg | S. Infantus | S.Typhimurium |
|------|----------|---------|--------------|-------------|---------------|
| 100% | 32 (70) | 33 (82) | 6 (12) | 6 (12) | 14 (46) |
| 90% | 7(70) | 6 (82) | 3 (12) | 2 (12) | 2 (46) |

Table 3: Frequency distribution of isolates by serotype and PFGE pattern phenotypic behavior. The phenotypic behavior describes that on the same day a 100% homologous PFGE pattern was isolated from pigs at slaughter and from the pen floor prior to the pigs being placed in the pen.

| Phenotype | S.Anatum | S.Derby | S.Heidelberg | S. Infantus | S.Typhimurium |
|-----------|----------|---------|--------------|-------------|---------------|
| No | 43 | 74 | 8 | 12 | 35 |
| Yes | 27 | 8 | 4 | 0 | 11 |

O 21 *Salmonella* surveillance trends in porcine *Salmonellae* in GB: 1996- 2002

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Summary: Comparison of serotype, phagetype prevalence and antimicrobial resistance profiles for 2002 with data from previous years shows an overall decrease in the number of *Salmonella* incidents in pigs. Nonetheless, the most frequently isolated serotypes remain unchanged, with an increase in *S. Typhimurium* incidents. The incidence of antimicrobial resistance for all *Salmonella* isolates from pigs during the study period showed increasing resistance trends to tetracycline and sulphamethoxazole/trimethoprim. However, the isolates remained sensitive to the majority of antibiotics in the screening panel.

Keywords: serotype, phagetype, antimicrobial resistance, human disease.

Introduction : This study describes the surveillance trends for *Salmonella* isolated from pigs in Great Britain over a seven-year period. The current use of in-feed antibiotics for both preventative and therapeutic purposes in livestock production has raised concern in relation to the emergence of antimicrobial resistant *Salmonella* from pigs. Trends for the major *Salmonella* serovars isolated from pigs are also considered. It is currently recognised that the majority of outbreaks during the summer months in England and Wales were primarily due to *Salmonella* infection linked to consumption of pig meat (Smerdon *et al*, 2001).

Materials and Methods: *Salmonella* isolates: A total of 2112 isolations of *Salmonella* submitted from 2048 recorded incidents, were received from the Veterinary Regional Laboratories in England and Wales during the period 1996-2002, were serotyped using a microtitre method based on the CPHL method (Shipp and Rowe, 1980). These *Salmonella* cultures were tested against 16 antimicrobial compounds.

Sensitivity tests: A disk diffusion technique using Sensitest agar (Oxoid) and antimicrobial containing disks (Oxoid) was used (Wray *et al.*, 1991). The disks contained the following antimicrobials: Amikacin (30µg) AK; Amoxicillin/clavulanic acid (30µg) AMC; Ampicillin, A (10µg); Apramycin, APR (15µg); Cefoperazone, CF (30µg); Cefuroxime, CX (30µg); Chloramphenicol, C (10µg); Chlortetracycline, T (10µg); Colistin, CT (25µ) Furazolidone, FR (15µg); Gentamicin, G (10µg); Nalidixic Acid, NA (30µg); Neomycin, N (10µg); Streptomycin, S (25µg); Sulphamethoxazole/trimethoprim, TM (25µg); Sulphonamide compounds, S3 (500µg, from 1998 onwards 300_µg was used). A growth inhibition zone diameter of less than 13mm was recorded as resistant (Sojka *et al.*, 1972).

Results: 1. *Serotype & phagetype prevalence isolated from pigs between 1996 and 2002.*

Salmonella Typhimurium was the most predominant serotype isolated from pigs during the 7 year study period, constituting between 59 % (1996) and 71 % (2002) of incidents. *Salmonella* Derby was found to be the next most commonly isolated serotype, contributing between 7 and 15 % of incidents over the study period. *S. Kedougou*, *S. Gold coast* and *S. Panama* constituted the other major serotypes most prevalent from porcine submissions.

The number of *Salmonella* Typhimurium DT104 incidents during the 7 year study period has decreased successively from 73 % of incidents in 1997 to 13 % in 2002. Interestingly, *S. Typhimurium* U308a has been isolated with increased frequency since 1999. The frequency of incidents attributed to *S. Typhimurium* DT 193 has remained consistent within the study period. Other *S. Typhimurium* incidents were mainly attributable to, U302, U288 and U308. During 2002, infection with *S. Enteritidis* was very low with only 1 incident of *S. Enteritidis* PT8.

2. *Antimicrobial resistance patterns of S. Typhimurium and other serotypes.*

Between 1996 and 2002 the majority of *S. Typhimurium* DT104 isolates from pigs showed the recognised resistance pattern AM, C, S, SU, T, with multiple antibiotic resistance (defined as \geq 4 antibiotics in the panel of 16 antibiotics tested) detected in DT104, DT104b, DT120, DT193, DT193a, DT208, DT7, DT12, U288, U302, U308a and U310.

Between 1996 and 2002 there was a marked increase in antimicrobial resistance of *S. Typhimurium* isolated to sulphamethoxazole/trimethoprim, with increasing resistance from 16 % in 1996 to 44 % in 2002. Many of the determinative phagetypes of *S. Typhimurium* isolated from pigs (DT193, DT208, U288, U308a, U310) have been shown to be resistant to sulphamethoxazole/trimethoprim.

The tetracycline resistance prevalence observed for *S. Typhimurium* appears to have sustained a consistently high trend with approximately 95 % of pig isolates being recorded as resistant in 2002. Corresponding resistance patterns to Aprimycin were consistently low whereas a resistance to Nalidixic acid was seen to rise slightly from >1 % in 1996 to 5.6 % in 2002.

The incidence of antimicrobial sensitivity for all *Salmonella* isolates excluding *S. Typhimurium* during the study period showed a rise in antimicrobial resistance. However, the isolates in the study were sensitive to the majority of antibiotics in the screening panel. Multiresistant strains of *S. Newport* have not been detected within the study period.

Conclusions: Predominant *Salmonella* serotypes and resistance patterns remained consistent over the 7 year study period. The incidence of antibiotic resistant *S. Typhimurium* in pigs in Great Britain has

been increasing despite a reduction in annual submissions. *S. Typhimurium* U308a has been isolated with increased frequency since 1999. The incidence of antimicrobial resistance for all *Salmonella* isolates from pigs showed increasing resistance trends to tetracycline and sulphamethoxazole/trimethoprim.

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O 22 Investigations of potential transfer of *Campylobacter coli* between hogs and turkeys.

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Summary: Hogs are often grown in close proximity to turkey farms in North Carolina, and the potential exists for transfer of pathogens, including *Campylobacter*, from one host animal to another. The aim of this study was to obtain evidence for possible transfer of *Campylobacter coli* from hogs to turkeys, or vice versa. Strains from four paired hog and turkey farms were isolated and characterized in terms of their antibiotic resistance profiles, and by molecular subtyping utilizing PCR-RFLP of *flaA*. Certain strains were found to be shared between hogs and turkeys, suggesting possible transfer. In spite of identical molecular subtypes, such strains commonly differed in antibiotic resistance profiles. The results are consistent with the hypothesis that strains of *C. coli* may transfer between hogs and turkeys, or that certain strain subtypes may independently colonize these animals through unidentified reservoirs.

Keywords: Strain subtypes, antibiotic resistance, PCR-RFLP, reservoir, prevalence

Introduction: *Campylobacter* spp., especially *Campylobacter jejuni* and *Campylobacter coli*, are recognized as leading bacterial causes of acute human gastroenteritis (Campylobacteriosis). *Campylobacter* is a zoonotic pathogen, which colonizes meat animals (poultry, hogs, cattle and others) and becomes transmitted to humans primarily through meat contaminated during slaughter and processing (Friedman et al., 2000).

Although various meat animals are known to be commonly colonized by campylobacters, a degree of host adaptation appears to exist. Poultry are most frequently colonized by *C. jejuni*, followed by *C. coli*, whereas cattle and swine are colonized almost exclusively with *C. jejuni* and *C. coli*, respectively (Aarestrup et al., 1997; Saenz et al., 2000; van Looveren et al., 2001). However, circumstantial evidence exists for possible transfers among hosts, and common strain types between *Campylobacter* from broilers and other animals (cattle, swine) have been reported (Aeschbacher and Piffaretti, 1989; Meinersman et al., 1997; On et al., 1998).

Our laboratory has been investigating the prevalence and strain types of *Campylobacter* from turkey flocks since 2001. We have found that turkey flocks in eastern North Carolina are frequently colonized by *C. coli*. The high frequency of *C. coli* in turkeys is of interest, as this bacterium is normally associated with swine (Aarestrup et al., 1997; Saenz et al., 2000). In other surveys of turkey colonization by *Campylobacter*, *C. coli* was found only in 9% of the isolates in one study (van Looveren et al., 2001), and not at all in another (Wallace et al., 1998).

Eastern N. Carolina is a major production region in the United States for both hogs and turkeys, and turkey husbandry often operates side-to-side with hog production. Although different service people and veterinarians typically serve turkeys and hogs, the potential exists for contact between the turkey and hog operations through the farmer, farm staff, and vehicular traffic (the farmer's truck etc).

The objective of this study was to investigate the potential transfer of *C. coli* from hogs to turkeys in hog-turkey production systems that operate in close proximity to each other. Four paired hog-turkey farms were examined in terms of the strain subtype and antibiotic resistance profile of *C. coli* that colonized the animals. Our results suggest the possibility of transfer of the organism from hogs to turkeys, with subsequent acquisition of different antibiotic resistance determinants.

Materials and methods: The turkey and hog farm pairs were typically within 100 m of each other, and each pair consisted of a growout turkey farm and a finishing hog farm. Fresh fecal samples were collected in sterile tubes, and transported to the laboratory on ice. Ca. 0.1 g from the interior of the sample was directly plated on a modified CCDA plate (Oxoid) and incubated at 42 AC microaerobically for 48 h. Purifications were on sheep blood agar plates (Remel). Antibiotic resistance determinations employed the disk diffusion test.

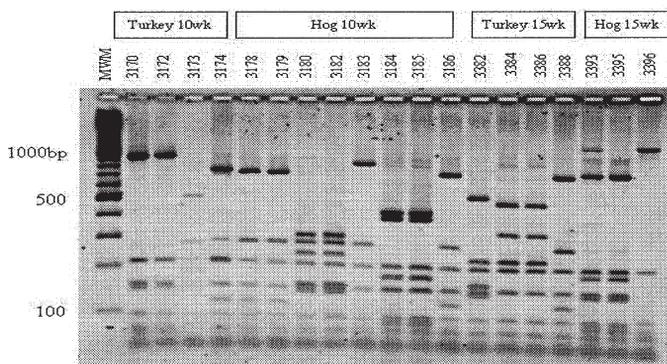
Genomic DNA was extracted with the Dneasy kit (Qiagen) and polymerase chain reaction (PCR) with primers for *hip* (Chan et al., 2000) and for *ceuE* (Gonzalez et al., 1997) was used to identify *C. jejuni* and *C. coli*, respectively. Molecular subtyping of the bacteria utilized PCR-restriction fragment length polymorphism (PCR-RFLP) of *flaA*, involving amplification of *flaA* and subsequent digestion with restriction endonuclease *DdeI* (Nachamkin et al., 1993). The restriction fragments were separated by agarose gel electrophoresis (1 % agarose in Tris Borate EDTA buffer) and photographed.

Results: All four pairs of hog-turkey production systems were found to be colonized by *Campylobacter* at high prevalence when sampled. Upon direct plating, *Campylobacter* was isolated from 50-85 % of the hog fecal samples, and from 55-95 % of the turkey samples. All *Campylobacter* strains from hogs were *C. coli*. Prevalence of *C. coli* among the turkey isolates was 68%, on the average (range among farms, 35 – 100 %), with the remainder being *C. jejuni*.

Resistance to tetracycline (30 mg/L) was uniformly high in both turkey and hog derived isolates of *C. coli* (93 and 96 %, respectively), and resistance to ampicillin (100 mg/L) was 75 % for *C. coli* from turkeys and hogs. However, significant differences were found in the incidence of ciprofloxacin and nalidixic acid resistance. *C. coli* strains from three of the hog farms were much less frequently resistant to these antibiotics (4, 31, and 18 %) than *C. coli* from the corresponding turkey farms (60, 67, and 60 %, respectively). Resistance was rare in the remaining farm pair.

Several different strain *flaA* strain subtypes of *C. coli* were isolated from the hogs. In each farm, several samples (20-60 %) were found to harbor bacteria of the same *flaA* type. Unique strain subtypes, encountered only once among the samples from each farm, and not shared among farms, constituted 11, 26, 32, and 35% of the strain subtypes. Although each of the four farms had its characteristic prevalent strain subtypes, one subtype, designated cc5, was encountered among all four farms (Table 1). However, strains of the same subtype frequently had different antibiotic resistance phenotypes, and generally the antibiotic resistance profiles of such strains differed from farm to farm.

Fig. 1. Strain subtypes of *C. coli* from a hog-turkey production system.



To evaluate the genetic similarity between strains from turkeys and from hogs, we employed *flaA* typing. Fig. 1 shows some of the strain subtypes of *C. coli* isolates from hogs of one farm, and of the corresponding turkey isolates. It can be seen that certain strain types were identical between hogs and turkeys (e.g. strains 3174 (turkey), 3178 (hog), 3179 (hog), 3186 (hog) and 3388 (turkey)). Interestingly, the antibiotic resistance profiles of the strains differed. The hog-derived strains were resistant to tetracycline, ampicillin, streptomycin and erythromycin, whereas the turkey-derived strains were additionally resistant to kanamycin, ciprofloxacin and nalidixic acid. It is worthy of note that the hog samples at the first time point (at which time the turkeys were 10 wk old) all had the same antibiotic resistance markers (resistance to tetracycline, streptomycin, ampicillin and erythromycin), even though they included 4 distinct strain subtypes, one of which (cc5, detected in 3 of the 8 strains) was also detected in the turkey samples.

Similar findings were obtained with the other farms. Although certain strains derived from hogs had identical strain types to those derived from the corresponding turkey farms, the antibiotic resistance profiles of the strains were usually different. Exceptions were occasionally noted. For instance, the hog-derived strain of subtype cc5 from farm 3 had a phenotype identical to several cc5 strains from turkeys, and was resistant to all antibiotics in the panel, including fluoroquinolones.

Discussion. The observed relatively high prevalence of *C. coli* in the turkeys is consistent with the hypothesis of transfer of this organism from hogs to turkeys. Since the antibiotic treatment regimens are distinctly different between hogs and turkeys, there would be selection for resistance to antibiotics used during turkey husbandry. In such a scenario, the strains would differ in antibiotic resistance but would be otherwise closely related genetically.

Although our data do provide evidence for strain subtypes that may be shared between hogs and turkeys in these production systems, further work is needed to confirm the putative transfer and determine its direction. *C. coli* typically colonizes hogs, which could therefore serve as a *C. coli* reservoir for other animals, such as turkeys. Turkeys have been described to be primarily colonized by *C. jejuni* (Wallace et al., 1998; van Looveren et al., 2001), and transfer from hogs, directly or indirectly, may account for the observed relatively high frequency of *C. coli* among the turkey samples in this study. Such transfer would be facilitated when turkeys and hogs are grown in close proximity, as was the case with the paired hog and turkey farms surveyed here. However, we have detected relatively high frequency of *C. coli* in turkeys from a number of different farms in eastern North Carolina, a hog-dense region (S. Kathariou and D. K. Carver, unpublished). One may speculate that, once turkeys become colonized by *C. coli*, the bacteria could transfer back to hogs. Results from such transfers may be evidenced by the isolation of strains with antibiotic resistance profiles typical of those of turkey-derived strains, such as the cc5 hog-derived strain from farm 3.

A key question that is generated by our findings concerns the origin of the shared strains. Further work is needed to determine the natural reservoir of strains with subtype cc5, which were found in both hog and turkey-derived strains in this study. Subtype cc5 has been repeatedly detected among *C. coli* isolates from turkeys from this geographical region, and, in addition, among *C. coli* recovered from flies in turkey and hog farms (B. C. Lee, L. Zurek and S. Kathariou, unpublished). Further work is needed to clearly determine whether these strains actually transfer from hogs to turkeys (and possibly vice versa) or whether hogs and turkeys receive them independently from other sources, that are currently not identified.

The results from this study identify some of the complexities of the ecology of *Campylobacter* in hog production, and are being followed by additional investigations in our laboratory, which are expected to elucidate some of the issues raised above.

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Table 1: Strain subtype distribution among *C. coli* from hog-turkey production systems. Strain subtypes indicated in bold were found among both hog and turkey-derived strains. Numbers in parentheses indicate the number of strains with the specific subtype.

| Farm 1 | | Farm 2 | | Farm 3 | | Farm 4 | |
|-----------------|------------|----------------|---------|----------------|------------|----------------|------------|
| Hogs | Turkeys | Hogs | Turkeys | Hogs | Turkeys | Hogs | Turkeys |
| Cc5 (7) | Cc5 (2) | Cc5 (3) | Cc5 (6) | Cc5 (1) | Cc5 (8) | Cc5 (4) | Cc5 (7) |
| GTH1 (2) | Cc4 (3) | GTH1 (4) | | GTA (15) | GTT1 (2) | Cc4 (1) | GTT3 (2) |
| GTH2 (5) | GTH3 (1) | Unique (5) | | GTB (2) | GTT2 (2) | GTA (1) | GTT4 (2) |
| GTH3 (3) | Unique (1) | | | Unique (7) | Unique (1) | GTC (2) | Cc4 (1) |
| Unique (2) | | | | | | GTH4 (3) | Unique (2) |
| | | | | | | GTH5 (2) | |
| | | | | | | GTH6 (2) | |
| | | | | | | Unique (3) | |

O 23

Comparison of two commercial ELISA kits and bacteriology for *Salmonella* monitoring in pig herds

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Summary: Samples of 'meat-juice' and serum from 170 pigs from 20 finishing farms were tested for *Salmonella* using two commercial ELISA kit tests. In parallel samples from caecal contents and pooled pen faeces from the farm were tested by culture. Both ELISA's gave significantly correlated results with each other but only ELISA B, at a 20 % calculated OD % on 'meat juice', gave a result which correlated significantly with the percentage of positive pen faeces. None of the ELISA tests correlated with caecal positives and the 10 % cut-off level was shown to be unsuitable for monitoring commercial herds.

Keywords: serology, pork, pigs, swine, culture

Introduction: Serological testing of pig herds for *Salmonella*, despite its epidemiological drawbacks, is the most widely accepted method of monitoring, largely on the grounds of convenience and cost. It is desirable that methods used for such testing should be as standardised as possible but an international ring trial has shown large differences in the performance of various tests (Heijden, 2001). The work carried out in this study was designed to evaluate two commercial ELISA kits for suitability of use for monitoring in British pig herds.

Materials and Methods: Serum and meat juice from 170 pigs originating from 20 British finishing herds were examined for anti-*Salmonella* antibodies using two commercial ELISA tests, A (Guildhay)

and B (IDEXX), according to manufacturers' instructions. In parallel 25 g caecal samples were collected from each pig and 25 g pooled faeces samples were taken from up to 20 pens on the farm of origin. Statistical analysis was carried out using Statistica Software. Samples were cultured using a BPW/Diasalm/Rambach agar method.

Results:

Tables 1 and 2 show a summary of results from each of the farms.

Salmonella, predominantly *S.Typhimurium*, was found in 47/200 (23.5 %) of caecal contents and in 121/369 (32.8 %) of pooled pen faeces. 26.5 % pigs gave a positive serum ELISA result with Kit A and 37.0 % with Kit B, at the 40 % OD level. At 20 % and 10 % OD Kit B gave 62.0 % and 81.0 % positive results respectively. Positive results for 'meat-juice' were 42.3 %, 38.2 %, 56.5 % and 71.2 %.

Using ELISA A for serum samples 61.7 % of caecal culture positives were test negative, compared with 46.8 % with ELISA B at 40 % OD. The comparable results for meat-juice were 36.2 % and 40.4 % for the 40 % OD level. 64.1 % serum positives by ELISA A were culture negative and 70.3 % by ELISA B. For meat-juice 60.2 % ELISA A positives were culture negative as were 66.1 % by ELISA B.

All of the ELISA tests correlated significantly with each other but the only correlation between culture and serology was for ELISA B at the 20 % OD level. This correlated significantly ($p < 0.05$) with the percentage of positive farm pen samples.

Discussion: This study has confirmed the difficulties involved in standardising test methodology for ELISA based monitoring of *Salmonella*. Standardisation and maintaining repeatability are problematic in that the quality of antigens and reference sera may change over time, especially when new batches of antigen are produced for the test. There is less of a problem if a single, well quality-controlled test is chosen for national surveillance and maintained over several years in a standardised way. It is usual however for economic forces or ongoing test improvements or other modifications to result in significant changes over time. Similarly, if different tests are in use and one country or region is being compared with another then more problem herds will be identified by those using more sensitive tests. These problems may also lead to disputes when a test results in a pig farm being placed in a *Salmonella* intervention category when another test may have given different results requiring no action. The same applies to inconclusive results, as recorded by ELISA A, which may require retests to clarify the herd *Salmonella* status designation. The work has also once more demonstrated a poor correlation between serological and bacteriological results. This is not necessarily a problem as herds are classified in broad bands associated with risk, and the GB approach of selecting the highest % positive test herds for further action is a logical one. The relationship of serology to bacteriology is likely to improve as herds are monitored over time and weighted rolling mean results can be applied (Steinbach, 2002). This should be studied by means of detailed longitudinal testing in British pig herds.

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ORAL PRESENTATIONS

Table 1: Comparison of Bacteriological and Serological Results using Two ELISA Kits

| Farm Ref | Caecal Contents Culture Positive | Serum | | | | | | | | |
|--------------|----------------------------------|----------------------------|----------------|-------------------|-------------------|------------------|--------------|--------------|------------|--------------|
| | | ELISA A | ELISA B 10% OD | ELISA B 20% OD | ELISA B 40% OD | ELISA - CAECAL+ | Both +ve | A Only | B Only | |
| 1 | 3 ^a /10 | 6/10 | 1 | 10/10 | 9/10 | 7/10 | 1 | 6 | 0 | 1 |
| 2 | 4 ^a /10 | 4/10 | 2 | 10/10 | 7/10 | 5/10 | 1 | 4 | 0 | 1 |
| 3 | 2 ^a /10 | 3/10 | 2 | 10/10 | 8/10 | 4/10 | 1 | 3 | 0 | 1 |
| 4 | 2 ^a /10 | 4/10 | 0 | 9/10 | 8/10 | 7/10 | 0 | 4 | 0 | 3 |
| 5 | 2 ^a /10 | 4/10 | 0 | 9/10 | 7/10 | 5/10 | 0 | 4 | 0 | 1 |
| 6 | 0/10 | 0/10 | 0 | 4/10 | 0/10 | 0/10 | 0 | 0 | 0 | 0 |
| 7 | 6 ^{ab} /10 | 2/10 | 5 | 9/10 | 6/10 | 2/10 | 4 | 1 | 0 | 1 |
| 8 | 0/10 | 1/10 | 0 | 1/10 | 1/10 | 1/10 | 0 | 0 | 1 | 1 |
| 9 | 2 ^a /10 | 2/10 | 1 | 7/10 | 4/10 | 2/10 | 1 | 2 | 0 | 0 |
| 10 | 2 ^{a,c} /10 | 2/10 | 2 | 9/10 | 7/10 | 3/10 | 2 | 2 | 0 | 1 |
| 11 | 4 ^a /10 | 0/10 | 4 | 8/10 | 6/10 | 1/10 | 3 | 0 | 0 | 1 |
| 12 | 6 ^a /10 | 0/10 | 6 | 5/10 | 3/10 | 1/10 | 5 | 0 | 0 | 1 |
| 13 | 0/10 | 0/10 | 0 | 8/10 | 3/10 | 2/10 | 0 | 0 | 0 | 2 |
| 14 | 3 ^a /10 | 5/10 | 0 | 8/10 | 6/10 | 5/10 | 0 | 5 | 0 | 0 |
| 15 | 0/10 | 3/10 | 0 | 6/10 | 6/10 | 3/10 | 0 | 3 | 0 | 0 |
| 16 | 0/10 | 1/10 | 0 | 10/10 | 7/10 | 2/10 | 0 | 1 | 0 | 2 |
| 17 | 2 ^d /10 | 4/10 | 2 | 9/10 | 8/10 | 4/10 | 2 | 4 | 0 | 0 |
| 18 | 6 ^{a,c} /10 | 2/10 | 4 | 10/10 | 9/10 | 5/10 | 2 | 2 | 0 | 3 |
| 19 | 3 ^a /10 | 3/10 | 0 | 10/10 | 9/10 | 7/10 | 0 | 3 | 0 | 4 |
| 20 | 0/10 | 7/10 | 0 | 10/10 | 10/10 | 8/10 | 0 | 7 | 0 | 1 |
| TOTAL | 47/200 (23.5) | 53/200 (26.5) | 29 (61.7) | 162/200 (81.0) | 124/200 (62.0) | 74/200 (37.0) | 22 (46.8) | 51 (67.1) | 1 (1.3) | 24 (31.6) |
| | | Total comparable +ves - 76 | | | | | | | | |

Key:

No. Samples positive / No. Samples tested (%)

Salmonella Serotype Key: ^a *S.*Typhimurium; ^b *S.*Newport; ^c *S.*Derby; ^d *S.*Indiana; ^e *S.*Enteritidis; ^f *S.*Agona; ^h *S.*Reading

A 34 (64.1%) serum +ve not caecal +ve

B 52 (70.3%) serum “

A 50 (60.2%) juice “

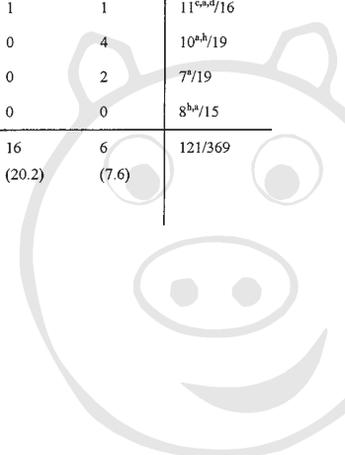
B 43 (66.1%) juice “

ORAL PRESENTATIONS

Table 2: Comparison of Bacteriological and Serological Results using Two ELISA Kits (cont'd)

| Farm Ref | Meat Juice | | | | | | | | | Farm Poole Faeces (% positive) |
|--------------|----------------------------|----------------|-------------------|------------------|------------------|--------------|--------------|--------------|------------|--------------------------------|
| | ELISA A | ELISA B 10% OD | ELISA B 20% OD | ELISA B 40% OD | ELISA - CAECAL+ | Both +ve | A Only | B Only | | |
| 1 | 6/10 | 1 | 7/9 | 6/9 | 2/9 | 1 | 2 | 3 | 0 | 2 ^a /20 |
| 2 | 6/10 | 1 | 9/10 | 7/10 | 6/10 | 1 | 6 | 0 | 0 | 9 ^a /20 |
| 3 | 6/10 | 1 | 9/10 | 6/10 | 5/10 | 1 | 5 | 1 | 0 | 5 ^a /20 |
| 4 | 6/10 | 0 | 8/9 | 6/9 | 5/9 | 0 | 5 | 1 | 0 | 0/15 |
| 5 | 9/10 | 0 | 8/9 | 6/9 | 5/9 | 0 | 5 | 4 | 0 | 7 ^{a,c} /17 |
| 6 | 0/10 | 0 | 0/10 | 0/10 | 0/10 | 0 | 0 | 0 | 0 | 0/20 |
| 7 | 5/8 | 0 | 2/3 | 2/3 | 0/3 | 2 | 0 | 2 | 0 | 14 ^{a,b,c} /19 |
| 8 | 0/10 | 0 | 0/5 | 0/5 | 0/5 | 0 | 0 | 0 | 0 | 6 ^b /20 |
| 9 | 3/10 | 1 | 1/7 | 1/7 | 0/7 | 2 | 0 | 1 | 0 | 2 ^a /20 |
| 10 | 3/10 | 1 | 8/9 | 6/9 | 3/9 | 1 | 3 | 0 | 0 | 1 ^a /15 |
| 11 | 2/10 | 3 | 2/6 | 2/6 | 1/6 | 3 | 1 | 0 | 0 | 4 ^a /20 |
| 12 | 5/10 | 3 | 5/10 | 4/10 | 3/10 | 4 | 3 | 1 | 0 | 5 ^a /20 |
| 13 | 2/10 | 0 | 2/6 | 1/6 | 0/6 | 0 | 0 | 0 | 0 | 2 ^a /17 |
| 14 | 5/10 | 0 | 9/10 | 7/10 | 5/10 | 0 | 5 | 0 | 0 | 12 ^{a,d} /19 |
| 15 | 6/10 | 0 | 6/10 | 6/10 | 5/10 | 0 | 5 | 1 | 0 | 15 ^a /20 |
| 16 | 2/10 | 0 | 8/10 | 4/10 | 2/10 | 0 | 1 | 1 | 1 | 1 ^a /18 |
| 17 | 5/9 | 2 | 9/9 | 8/9 | 5/9 | 2 | 4 | 1 | 1 | 11 ^{a,c,d} /16 |
| 18 | 1/10 | 4 | 10/10 | 8/10 | 5/10 | 2 | 1 | 0 | 4 | 10 ^{a,b} /19 |
| 19 | 4/9 | 0 | 8/8 | 6/8 | 6/8 | 0 | 4 | 0 | 2 | 7 ^a /19 |
| 20 | 7/10 | 0 | 10/10 | 10/10 | 7/10 | 0 | 7 | 0 | 0 | 8 ^{b,a} /15 |
| TOTAL | 83/196 (42.3) | 17 (36.2) | 121/170 (71.2) | 96/170 (56.5) | 65/170 (38.2) | 19 (40.4) | 57 (72.1) | 16 (20.2) | 6 (7.6) | 121/369 |
| | Total comparable +ves - 79 | | | | | | | | | |

See Key above



Discrimination of Vaccinated and infected Pigs by *Salmonella*-specific IgA antibodies

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Summary: Isotype switching from IgM to IgG or IgA leads to generation of high affinity antibodies during the immune response. This phenomenon can be applied for differentiation of early and late infection stages. The purpose of this study was to evaluate the applicability of a newly developed Ig-isotype specific *Salmonella* antibody ELISA for discrimination between vaccinated and infected pigs. Using this novel ELISA we were able to detect different patterns of *Salmonella*-specific IgM, IgG and IgA antibodies following immunization with a *S. Typhimurium* live vaccine or after experimental infection with a nalidixic-acid resistant wild-type strain of *S. Typhimurium*. Interestingly, *Salmonella*-specific IgA antibodies represented an excellent tool for the recognition of fresh infection in vaccinated pigs. Under SPF conditions, we were able to discriminate between naive, vaccinated, experimentally infected nonvaccinated, and experimentally infected vaccinated animals. However, the highest specific IgA levels were detected in challenged vaccinated pigs. Preliminary results from field trials support the findings from experiments using SPF animals.

Keywords: Serology, Diagnostics, ELISA, Ig class, Vaccination

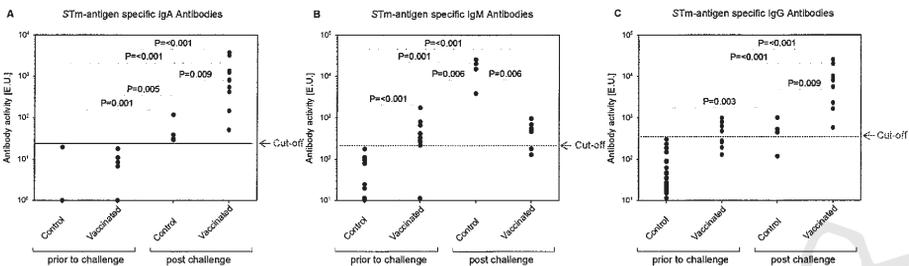
Introduction: Due to intensified stockbreeding the risk of *Salmonella* transmitted to man by consumption of animal food products increased. Vaccination with live attenuated vaccines represents an effective tool for reduction of *Salmonella* burden of pig herds. Nevertheless, farmer often refuse live vaccines being afraid to be unable to distinguish between antibody formation following the vaccination or infection with wild strains. Unfortunately, there exists no reliable assay to discriminate vaccinated from infected pigs so far which could overcome this dilemma. Therefore, we attempted to apply a novel isotype-specific ELISA system for discrimination between infected pigs and pigs immunized with a *S. Typhimurium* live vaccine.

Materials and Methods: The ELISA developed for this purpose uses antigens from whole cellular extract of *S. Typhimurium* (SALMOTYPE[®] Pig WCE-ELISA, Labor Diagnostik Leipzig, Leipzig, Germany). Antigen-bound IgM, IgG, and IgA antibodies were detected in swine serum samples by isotype-specific peroxidase-conjugated secondary antibodies. Sera derived from *S. Typhimurium* vaccination/challenge experiments using SPF pigs (n=4-20) and two different field trials were analysed for evaluation of the test. In order to minimize interassay variances and to normalize the measured signals any individual serum was log₂ diluted over four steps and compared to a defined reference standard serum which was equally diluted (Butler, 1991). For each Ig class (IgM, IgA, IgG) an especially calibrated reference standard serum was used. OD raw data were calculated into ELISA units (E.U.) by using a new software developed for this purpose (SALMOSoft[™], Labor Diagnostik Leipzig). The calculated E.U. were plotted statistical significances were calculated by using the SigmaStat[™] software (SPSS Science, Erkrath, Germany).

Results: Using the novel Ig isotype-specific ELISA we were able to detect different patterns of *Salmonella*-specific IgM, IgG and IgA antibodies following oral immunization with a *S. Typhimurium* live vaccine (SALMOPORC[™] IDT, Rosslau, Germany) or experimental infection with a nalidixic-acid resistant wild-type

strain of *S. Typhimurium* DT104 (958/96). Interestingly, *Salmonella*-specific IgA antibodies represented an excellent tool for the recognition of fresh infection in vaccinated pigs but to a lower extent also in nonvaccinated control animals. Thus, under SPF conditions, we were able to differentiate between naïve, vaccinated, experimentally infected nonvaccinated, and experimentally infected vaccinated pigs. However, the highest specific IgA levels were detected in challenged vaccinated pigs (Fig. 1A). Although the IgM values were found to be very high at day 7 post infection in nonimmunized pigs, which recommends IgM as an indicator of very early infection in nonvaccinated pigs, IgM is not a reliable parameter for *Salmonella* infections in vaccinated pigs since there was an overlapping region with the IgM levels of healthy, nonimmunized controls animals (Fig. 1B). A similar result was observed in terms of IgG. But in contrast to IgM, *Salmonella*-specific IgG was an appropriate indicator of the secondary immune response induced by an infection in immunized pigs similar to IgA, but did not clearly identify the vaccine induced primary immune response since the half of the values overlapped with those of control animals (Fig. 1C). In general, the background IgG level was higher than the background levels of IgM and IgA. Therefore, IgA revealed to be the most appropriate parameter for our purpose. Preliminary results from field trials confirm the findings from experiments using SPF animals. Taken together, these data provide a novel approach for the identification of *Salmonella*-infected pigs and the discrimination from pigs immunized with *Salmonella* live attenuated vaccines.

Figure 1: Recognition of *S. Typhimurium* infected SPF pigs by detection of *Salmonella*-antigen specific IgM, IgG, and IgA antibodies. IgA was most appropriate for discrimination of infected animals. Statistical significances were calculated by using the Mann-Whitney rank sum test.



Discussion and Conclusion: The evaluation of *Salmonella* incidence in pig herds or even individual animals, particularly in breeding facilities, by serological tests has been a matter of discussion for many years. However, the high sensitivity represents an important advantage of serological compared to bacteriological diagnostic methods. A disadvantage of many serological tests is their low specificity which depends mainly on the nature of the antigen used. As demonstrated in this study the use of whole cellular extract antigen in combination with the detection of certain Ig isotypes allows the recognition of *Salmonella* infection in pigs with relatively high precision. This novel test offers a hopeful tool not only for highly reliable evaluation of *Salmonella* incidence in pig herds or the infection and/or immune status of individual animals but also to promote the acceptance of immunization of pig herds using live vaccines under diagnostic control.

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O 25 EXPRESSION STUDY BY REAL-TIME QUANTITATIVE RT-PCR OF THE *SALMONELLA* TYPHIMURIUM *mntH* GENE

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Summary: The aim of our study was to compare the *mntH* expression of different *Salmonella* Typhimurium strains and other *Salmonella* serovars with real-time RT-PCR. Following the *mntH* expression in function of the growth showed that the *mntH* expression of *S. Typhimurium* is growth dependent. A strong decrease of the *mntH* expression is noticed when the growth reaches $1.78 \cdot 10^8$ CFU/ml. After induction with EDTA or H_2O_2 , variations between different *S. Typhimurium* strains were observed. For some *S. Typhimurium* strains a 10 to 20 times higher *mntH* expression was noticed after H_2O_2 induction. The EDTA induction was for most strains lower (5 to 10 times) but also variations between different strains were observed. The other *Salmonella* serovars were strongly induced after H_2O_2 but not after EDTA induction.

Introduction: The NRAMP (natural resistance-associated macrophage protein) family of divalent-metal transporters was first identified by studies in mice on the genetic basis of susceptibility to intracellular parasites. The NRAMP family is widespread and highly conserved with homologues in other animals, insects, worms and plants. In bacteria an orthologue of the eucaryotic *Nramp1* gene is described, the *mntH* gene (H^+ -coupled manganese transport). In the phagosome the bacterial and the eucaryotic NRAMP would compete for the transport of bivalent cations. The competition between both transport systems would determine the intracellular survival of the pathogenic bacterium (Agranoff et al, 1999). Differences in the activity of the *mntH* gene could be important for the pathogenicity of the strain and the capacity to survive in the host. In our study the expression of the *mntH* gene was compared from different pig related *Salmonella* Typhimurium strains after induction with EDTA and H_2O_2 .

Materials and methods: 30 pig related *S. Typhimurium* strains of different origin, an *mntH* deletion mutant of *S. Typhimurium* MB 2534 and 2 human *S. Typhimurium* strains were selected for this study. To compare with other *Salmonella* serovars a *S. Enteritidis*, *S. Kedougou*, *S. Havana*, *S. Ohio*, *S. Livingstone*, *S. Derby*, *S. Goldcoast* and *S. Brandenburg* were included. **Determination of the growth curve:** For 3 *S. Typhimurium* strains MB 2150, MB 2486, MB 2109 and an *S. Derby* strain 821175a the growth curve was determined in BHI at 37°C. Starting from $1.0 \cdot 10^6$ CFU/ml, every 30 minutes a 1 ml sample was taken and total RNA was extracted using the RNeasy Mini kit (Qiagen) as described by the manufacturer. Residual DNA was digested using a DNase treatment. The amount of *mntH*, 16S rRNA and *rpoD* mRNA was measured. For the induction experiment strains were grown to OD 0.050. To the bacteria culture H_2O_2 (100 μ M) or EDTA (1 mM) was added. After H_2O_2 induction the sample was taken directly (T0) whereas for EDTA induction a sample after 15 and 45 minutes incubation at 37°C was taken. Reverse transcription was carried out with random hexamers and Multiscribe Reverse Transcriptase (Applied Biosystems) with 1 μ g RNA as template. 5 μ l of the cDNA was used in the real-time PCR amplification mixture containing 1x SyberGreen I master mix (Applied Biosystems) and 300 nM of the forward and reverse primer. As control genes the 16S rRNA and the *gmk* (guanylate kinase gene) were analyzed.

Results: The *mntH* expression of *S. Typhimurium* is growth dependent. Figure 1 presented the expression in function of the growth in BHI for *S. Typhimurium* strain MB 2486. The same results were obtained with the other strains (results not shown). A strong decrease of the *mntH* expression is noticed when the OD₆₁₀ of the culture is greater than 0.5. The expression of 16S rRNA remains constant during growth and a decrease in *rpoD* expression is noticed when the plateau phase is reached.

After induction with EDTA or H₂O₂, variations between different *S. Typhimurium* strains were observed. For some *S. Typhimurium* strains a 10 to 20 times higher *mntH* expression was noticed after H₂O₂ induction. The EDTA induction was for most strains lower (5 to 10 times) but also variations between different strains were observed. The other *Salmonella* serovars were strongly induced after H₂O₂ but not after EDTA induction. In the *mntH* mutant no detectable level of *mntH* mRNA could be measured after a 40 cycle PCR.

For the relative quantification *rpoD* could not be used as control gene because it turned out to be induced by H₂O₂. The relative expression of the *mntH* gene normalized against both control genes 16S and *gmk* was comparable.

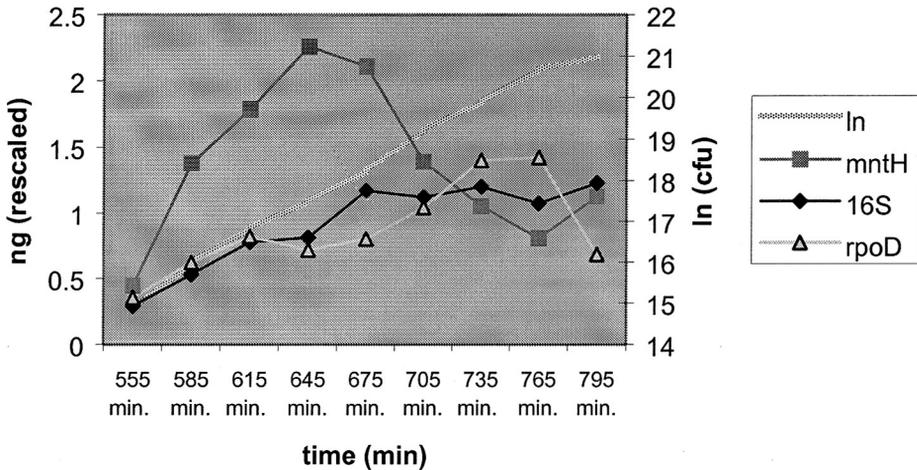


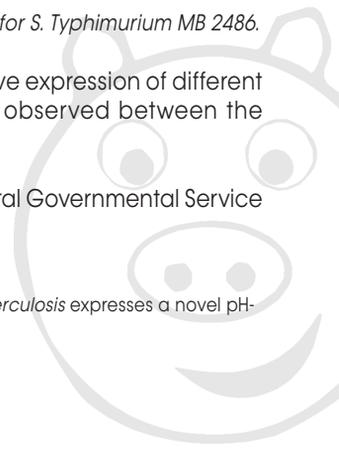
Figure 1: The expression of *mntH*, 16S rRNA and *rpoD* in function of the growth in BHI for *S. Typhimurium* MB 2486.

Conclusion: Real time PCR is an efficient tool to study the relative quantitative expression of different genes. By these technique differences in the *mntH* expression could be observed between the different *S. Typhimurium* strains.

Acknowledgement: This work was financially supported by the Belgian Federal Governmental Service of Public Health, Safety of the Food Chain and Environment.

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Development of a new molecular typing method of *Salmonella* spp. based on SNPs detection

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Summary: The aim of this study was to develop a new molecular typing method of *Salmonella* spp. based on the detection of point mutations (Single Nucleotide Polymorphisms or SNPs) in the genome. Several genomic regions (*ManB*, *InvA*, *pduF*) were sequenced in strains isolated from pig, the sequences were aligned and 49 point mutations were identified. A panel of 12 SNPs giving a genotype characteristic of a particular serotype was selected. Until now, these selected SNPs were unable to distinguish the all strains. Therefore, additional SNPs must be selected.

Keywords: genotyping, point mutations, automation.

Introduction: *Salmonella enterica* is a common cause of foodborne intoxication. Pig is a major reservoir of these pathogenic bacteria. Our previous study indicated that the prevalence on carcasses is around 20 %. Since *Salmonella enterica* contains many different serotypes of different pathogenicity, epidemiological studies require very powerful tools. Until now, the molecular typing methods include: ribotyping, pulse field gel electrophoresis (PFGE) (Fontana et al., 2003), Amplified Fragment Length Polymorphism (AFLP) (Hu et al., 2002), Random Amplification of Polymorphic DNA (RAPD) (Khooodoo et al., 2002) or Multi Loci Sequencing Typing (MLST) (Kotetishvili et al., 2002). All these methods are indirect ways to study the point mutations occurring in the bacterial genome during evolution. These point mutations (one base replaces another) referred to Single Nucleotide Polymorphism (SNP) that are widely used in eukaryotes as genetic markers. The aim was to select SNPs allowing to distinguish one strain from another by using an automated genotyping method.

Material and methods: Strains. Three strains of the following serotypes were used: *S. thyphimurium*, *S. thyphimurium copenhagen*, *S. Livingstone*, *S. infantis*, *S. mbandaka*, *S. london*, *S. bovismorbificans*, *S. goldcoast*, *S. bochum*, *S. anatum*, *S. nigeria*, *S. rissen*, *S. panama*, *S. ibadan*, *S. africana*, *S. enteritidis*, *S. odozi*, *S. virchow*, *S. babelsberg*, *S. agona*, *S. adjame*, *S. lexington*, *S. Brandenburg*, *S. maritzburg*. They were all isolated from pig samples (meat, feces). PCR. The DNA was extracted using a commercial kit (Wizard genomic kit, Promega). The PCR primers were selected using the Oligo6 software (Medprobe). The PCR were performed using the following mastermix: ADN (10-100 ng), 1 unit of Taq DNA polymerase (Amersham Biosciences), 2 ml of 10 x buffer (500 mM KCl, 15 mM MgCl₂, and 100 mM Tris-HCl (pH 9)), 0,2ml of each primers (40 mM), 2 ml of 2 mM dNTPs and sterile pure water up to 20 ml. The cycles used were 1 times 94°C for 5 min., 40 times (94°C for 30 sec., OAT (optimal annealing temperature as calculated by the oligo6 software) for 30 sec, 72°C for 30 sec), 1 times 72°C for 5 min. The PCR products were purified using ExoSap-IT procedure (Amersham Biosciences). Sequencing. The Purified PCR products were sequenced using upper and lower PCR primers following the DYEnamic ET terminator cycle sequencing kit (Amersham Biosciences). The sequencing products were analyzed by capillary electrophoresis (Megabace 500, Amersham Biosciences). The deduced sequences were aligned using the Clustalw software (<http://www.ebi.ac.uk/clustalw/>). Genotyping. The PCR products were pooled and diluted ten times with water. Ten microliters were mixed with 5 ml of molecular weight marker (ET-550, Amersham Biosciences). The samples were analyzed by capillary electrophoresis (Megabace 500). The genotypes were deduced using the "Genetic Profiler" software (Amersham Biosciences).

Results: From genomic DNA, several genomic regions were amplified by PCR and sequenced. The sequences were aligned and the SNPs identified. The results were summarized in table 1. Among the

49 described SNPs, 12 were selected because they gave a genotype characteristic of the serotype. Allele specific primers were selected (table 2) and a multiplex PCR was performed in order to generate amplicons of different sizes. The mixture of amplicon was then analyzed by capillary electrophoresis. The size gave the selected SNP and the detected color gave the allele present. Nevertheless, the SNPs selected at the moment were unable to discriminate each strain. Therefore, the investigations to look for new SNPs were still running.

Discussion: In order to be able to evaluate the genetic linkage between Salmonella strains, it is important to be able to have a powerful genotyping method. Most of the existing methods were difficult to automate and have problems of repeatability and reproducibility (Bagley et al., 2001). Since most of the methods were based on the presence of point mutations, we chosen to directly detected the SNPs. The first step was to select SNPs by sequencing some genomic regions in several strains. This approach allowed us to select SNPs that are specific to a particular serotype. An automate genotyping method was developed to identify the Salmonella serotype. Nevertheless, it was actually not possible to distinguish the all strains. Therefore, we are investigating other polymorphic genomic regions. We focused our attention to intergenic regions and polymorphic regions selected by RAPD or AFLP.

Table 1. SNPs detected in Salmonella genomic regions

| Gene | primers | Amplicon size | N° of SNPs |
|------|---|---------------|------------|
| PduF | cgccagcctcggattat aacggcaaccagtacccc | 210 bp | 3 |
| ManB | ccggcaccgaagaga cgccgccatccggtc | 660 bp | 32 |
| InvA | gatttcctgategcactg ttggcggcgctac | 866 bp | 14 |

Table 2. Primers used for the serotype specific genotyping

| primers | dye | Amplicon size | primers | dye | Amplicon size |
|----------------------------------|------------------|---------------|---------------------------------|------------------|---------------|
| InvA114 Inva525G Inva525A | No FAM TET | 431 bp | ManB121C ManB121T ManB388 | FAM TET No | 287 bp |
| InvA662C InvA662T InvA1138 | FAM TET No | 496 bp | ManB295C ManB295T ManB619 | FAM TET No | 344 bp |
| InvA593C InvA593T INVA766 | FAM TET No | 189 bp | ManB256C ManB256T ManB618 | FAM TET No | 380 bp |
| InvA769 InvA906A nvA906A | No FAM TET | 157 bp | ManB165 ManB557C ManB557T | No FAM TET | 412 bp |
| InvA491C InvA491T InvA875 | FAM TET No | 399 bp | ManB242G ManB242T ManB686 | FAM TET No | 464 bp |
| ManB363 ManB461G ManB461A | No FAM TET | 114 bp | ManB258G ManB258C ManB719 | FAM TET No | 481 bp |

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O 27

CORRELATION BETWEEN BACTERIOLOGY OF LYMPH NODES AND SEROLOGY FOR *SALMONELLA* DIAGNOSIS IN SLAUGHTER PIGS

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Summary: *Salmonella* control programs in pigs are usually based on serological tests. The major objective of this cross-sectional study was to investigate the correlation between the serological results and the bacteriological results of *Salmonella* diagnosis in pigs at the herd level and at the animal level. From 60 farrow-to-finish herds, serum samples and mesenteric lymph nodes from 30 fattening pigs were taken in the slaughterhouse. Antibodies were determined using an indirect mix-ELISA. Qualitative *Salmonella* isolation was carried out on the lymph nodes. From a representative number of isolates, serotypes were determined. At the herd level, a Spearman's rank correlation coefficient of 0.28 was calculated ($p = 0.03$). The OR between serology and bacteriology based on individual samples was 1.54 (0.83 - 2.24). Associations were found between the serological and the bacteriological diagnosis, although not significant.

Keywords: *Salmonella* isolation, mix-ELISA, serotypes, odds ratio

Introduction: *Salmonella* in pork is one of the major sources for human salmonellosis. Most surveillance programs are based on serological tests carried out on blood samples taken at the slaughterhouse. It is important to know if serologically negative animals still can be carriers of *Salmonella*. The aim of present study was therefore to investigate the correlation between the serological and the bacteriological results for *Salmonella* diagnosis in blood samples and mesenteric lymph node samples taken at the slaughterhouse, at the individual level.

Materials and methods: Sixty farrow-to-finish pig herds participated in this study. From each herd, 30 randomly selected pigs were sampled at the slaughterhouse. Individually numbered pigs were blood sampled at killing, mesenteric lymph nodes were collected from the same pigs after evisceration. Blood samples were transported to the laboratory where antibodies were determined in an indirect

mix-ELISA combining the O-antigens 1, 4, 5, 6, 7, 9, 12 and 14 (Idexx). Samples were determined as positive if the OD% was higher than 10%. Lymph nodes were collected in sterile bags and transported to the laboratory under refrigerated conditions. All lymph node samples were submitted to qualitative *Salmonella* isolation, using standard procedures. From a maximum of 20 samples per herd, one *Salmonella* isolate per sample was further identified. Identification at serotype-level was first performed by the polymerase chain reaction (PCR) assay using the *Salmonella* Typhimurium-specific primers MDH 31 and MDH 2 coding for malic acid dehydrogenase designed by Lin et al. (1999). Isolates tested negative in the PCR-assay were serotyped according to the Kauffman-White Scheme, which uses O- and H-antigens (Popoff and Le Minor, 1992).

The association between serology and bacteriology at the herd level was quantified by means of a Spearman's rank correlation coefficient and at the individual pig level by means of odds ratio (OR). Pen was included as random effect for the analysis at the individual level (PROC NL MIXED SAS 8.02) because clustering is present when analysing results on pig level (Lo Fo Wong, 2001).

Results: The mean percentage (\pm SD) of positive samples per herd was 76.85 ± 24.0 % and 57.1 ± 36.6 % for the serology and the bacteriology, respectively. At the herd level, the correlation coefficient between serology and bacteriology was 0.28 ($p = 0.03$). An animal detected as serologically positive has more chance to be bacteriological positive than a serologically negative animal with an OR of 1.54 (0.83 - 2.24) ($p = 0.07$). A serologically negative animal has less chance to be bacteriological positive, with an OR of 0.65 (0.35 - 0.95) ($p = 0.07$). The serological results of the 12 most occurring serotypes are given in table 1.

Discussion: The existing control programs for *Salmonella* in pigs in Denmark and Germany are using serological tests on meat-juice samples (Mousing et al., 1997; Osterkorn et al., 2001). However, major sources for carcass contamination are *Salmonella* carriers and *Salmonella* shedding animals. To be able to draw conclusions based on serological results, it is important to know if serologically negative animals can still be carriers of *Salmonella* and thus be a hazard for human health. Associations between serological and bacteriological diagnosis at the herd level are weak but significant. As seen in the results of the present study, a serologically negative animal has less chance to be bacteriological positive compared to a serologically positive animal, although not significant. This means that there is a trend that *Salmonella* carriers are detected by the serological tests used, although there still are some discrepancies between both tests. The majority, but not all of the isolated serotypes are detected by the indirect ELISA used. Since seroconversion is first detectable between 3 and 14 days - depending on the serotype - after an experimental infection, (Idexx, personal communication), it is possible to isolate *Salmonella* when the animal is serologically negative. On the other hand, animals which are no longer carriers can still be serologically positive. The discrepancies between serological and bacteriological diagnosis should be taken into account when designing control programs to protect human health where rapid isolation techniques or PCR-tests would be more appropriate.

Table 1: Serological results for *Salmonella* per isolated serotype

| Serotype | Number of isolates | Serologically positive | Serologically negative |
|---------------------|--------------------|------------------------|------------------------|
| S. Typhimurium | 155 | 138 (89.0%) | 17 (11.0%) |
| S. Derby | 112 | 90 (80.4%) | 22 (19.6%) |
| S. Goldcoast | 44 | 38 (86.4%) | 6 (13.6%) |
| S. Livingstone | 29 | 26 (89.7%) | 3 (10.3%) |
| S. Panama | 25 | 18 (72.0%) | 7 (28.0%) |
| S. London | 19 | 13 (68.4%) | 6 (31.6%) |
| S. Brandenburg | 17 | 16 (94.1%) | 1 (5.9%) |
| S. Rissen | 17 | 16 (94.1%) | 1 (5.9%) |
| S. Urbana | 9 | 9 (100%) | 0 (0%) |
| S. Bovismorbificans | 8 | 7 (84.5%) | 1 (15.5%) |
| S. Infantis | 8 | 5 (62.5%) | 3 (37.5%) |
| S. Anatum | 7 | 6 (85.7%) | 1 (14.3%) |
| Total | 450 | 382 (84.9%) | 68 (15.1%) |

Conclusions: At the individual level, associations were found between the serological and the bacteriological diagnosis for *Salmonella*, although they are not significant. These discrepancies should be taken into account when designing *Salmonella* control programs based on serology.

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Effect of different enrichment media and DNA extraction techniques on *Salmonella* detection by PCR in SWINE feces

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Summary: The aim of this study was to evaluate different selective enrichment broths and DNA

extraction techniques on the detection of *Salmonella* Typhimurium in swine feces by PCR. Feces samples (n=10) were inoculated with approximately 10^2 *Salmonella* Typhimurium organisms, first enriched in GN-Hajna broth and secondly enriched in Rappaport-Vassiliadis, Muller-Kaufmann tetrathionate and selenite-cystine broths. In order to produce DNA-templates for PCR, aliquots from the broths were subjected to three DNA extraction methods: boiling-centrifugation, salting-out and phenol-chloroform. Detection of *Salmonella* was significant lower when phenol-chloroform was applied to selenite-cystine and Muller-Kaufmann tetrathionate ($P<0.05$). The boiling-centrifugation technique had best cost/benefit ratio and can be successfully used as a rapid DNA template preparation from the three enriched broths tested.

Keywords: Detection, enrichment, media, pigs, stool

Introduction: Polymerase chain reaction (PCR) based-methods for detection of *Salmonella* from clinical specimens have been of great interest to researchers considering it is pointed out to be faster and more sensitive than standard bacteriological procedures. However, many PCR-inhibitory substances are present in clinical samples, specially in feces. The use of PCR coupled to selective enrichment media has been shown to increase considerably the sensitivity of the PCR as they allow the increase of the *Salmonella* organisms in the sample and dilute inhibitors. However, some enrichment media have also been pointed out to inhibit PCR as well. The aim of this study was to evaluate different selective enrichment broths and DNA extraction techniques on the detection of *Salmonella* Typhimurium in swine feces by PCR.

Material and Methods: Feces samples from ten sows from a herd in which no *Salmonella* was isolated previously were collected, homogenised and subdivided in two ten-gram parcels. One of them was inoculated with *Salmonella* Typhimurium organisms (100 organisms/g) while the other received sterile media. The samples were first enriched in GN-Hajna broth (1:10) and secondly enriched in Rappaport-Vassiliadis (RV, 1:100), Muller-Kaufmann tetrathionate (TT,1:10) and selenite-cystine (SC, 1:10) broths. After incubation, aliquots were streaked onto XLT4 plates, which were incubated overnight at 37AC. Aliquots from the broths were subjected to three DNA extraction methods: boiling-centrifugation (M1), salting-out (M2) and phenol-chloroform (M3). In M1 (Soumet et al., 1994), an aliquot (1 mL) was centrifuged (13,000g, 3 min), the pellet resuspended in sterile bi-distilled water (100 mL) and boiled (95 AC, 10 min). The samples were finally centrifuged (8,000g, 3 min) before use. The salting-out technique for DNA precipitation (M2) was performed using a commercial kit (PuregeneB, Gentra). For M3, a protocol described by Oliveira et al. (2002) was used. Shortly, aliquots of 1 mL were centrifuged (2,000g, 4 min) and the pellet was resuspended in TE (10mM Tris-HCL pH 8, 1mM Na_2EDTA), 25 mL 10%SDS and proteinase K (20 mg/mL) and incubated (55AC, 30 min). Afterwards, phenol-chloroform pH 8 was added (1:1) and the samples centrifuged (10,000g, 4 min). The DNA was precipitated with 3M sodium acetate and cold isopropanol. The samples were centrifuged (16,000g, 10 min) and the pellet washed with 80% ethanol. The final pellet was resuspended in 50 mL of TE.

The PCR amplification mixture (25mL) consisted of MgCl_2 (2 mM), dNTP (50 mM of each deoxynucleoside triphosphate), primers (0.4 pmol of each) and Taq DNA polymerase (1 U). A primer set (S18 and S19) was used for genus specific detection of *Salmonella* (Kwang et al., 1996).

The comparative effectiveness among the selective broths on *Salmonella* detection by PCR was evaluated by a test to compare binomial proportions.

Results: *Salmonella* Typhimurium was recovered from all ten samples enriched in TT and only from four samples enriched in each RV and CS. The results from PCR are shown in Table 1. Detection of *Salmonella* was significant lower when M3 was applied to SC and TT ($P<0.05$).

Table 1. *Salmonella* Typhimurium detection by PCR in ten porcine stool samples artificially inoculated with 10² CFU/g. The methods for DNA isolation from feces were boiling-centrifugation (M1), salting-out (M2) and phenol-chloroform (M3) and were applied to the enriched broths selenite-cystine (SC), tetrathionate Muller-Kauffmann (TT) and Rappaport-Vassiliadis (RV).; *: Significant difference (P<0.05).

| Sample | M1 | | | M2 | | | M3 | | |
|--------------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|------------|-----------|
| | SC | TT | RV | SC | TT | RV | SC | TT | RV |
| 1 | + | + | + | - | + | + | + | - | + |
| 2 | + | + | + | + | + | + | + | - | + |
| 3 | + | + | + | - | + | + | - | - | + |
| 4 | + | + | + | + | + | + | - | - | - |
| 5 | - | + | + | + | + | + | - | - | - |
| 6 | + | + | + | + | + | + | - | - | + |
| 7 | + | + | + | + | + | + | - | - | + |
| 8 | - | + | - | - | + | - | - | - | - |
| 9 | + | + | - | + | - | + | - | - | + |
| 10 | + | + | + | + | + | + | - | - | + |
| Total | 08 | 10 | 08 | 07 | 09 | 09 | 02 | 00* | 07 |

Discussion: According to the results, RV broth seemed to be suitable to the three evaluated DNA extraction techniques. PCR effectiveness was lower (P<0.05) when either TT or SC were used for DNA-template preparation by the phenol-chloroform technique. Selenite-cystine broth has been pointed out to be less inhibitory than TT and RV (Stone et al., 1994). However, many reports describe the direct use of enrichment broths as DNA-template. Similar results between selenite and Rappaport-Vassiliadis broths were reported by Kongmuang et al. (1994).

Considering the higher microbiological isolation rate provided by the TT broth, the good PCR results seen in this medium were possibly affected by the high number of *Salmonella* yielded.

The remarkable speed and low cost of the boiling-centrifugation technique must be emphasised. Furthermore, we have demonstrated that RV, SC and TT can be successfully used as source of DNA for PCR. This information is valuable since complex DNA extraction methods are expensive, time-consuming and laborious. Finally, SC and TT broths must be avoided when DNA extraction is performed with phenol-chloroform.

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Development of an in vitro method for detection of *Clostridium botulinum* types A and E using real-time PCR

O 29

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Summary: Specific primers for *C. botulinum* types A and E neurotoxin genes were evaluated both from the literature and of own construction. Furthermore, a real-time system with specific hybridisation probes was designed for *C. botulinum* type E neurotoxin gene, and is under construction for type A. Sequencing of part of the neurotoxin gene for type E showed some differences with sequences reported in GenBank. The PCR methods were optimised regarding amplification efficiency, linear range and specificity. The detection limit for type E using real-time PCR is at least 0,1 ng/ml, corresponding to 0,4 pg of total DNA in the tube, and at least 0,5 ng/ml (2,5 pg of total DNA) for type A using conventional PCR. Quantitative reverse transcription PCR was used to study the relative expression of the neurotoxin gene in different growth phases.

Keywords: Botulism, neurotoxin, molecular methods, relative quantification, gene expression

Introduction: *C. botulinum* types A, B and E are the most common causes of foodborne botulism in humans. They are a diverse group of organisms that have different growth characteristics and nutritional requirements. The diagnosis of botulism is usually established by detecting the neurotoxin in serum, stomach content, faeces and suspected foods using the mouse bioassay. This method is, however, expensive, relatively slow and raises moral issues about animal rights. The use of PCR has been found to be an accurate and sensitive way to determine the presence of different foodborne pathogens. The ability to quickly and easily detect the bacteria and its neurotoxin would increase the possibility of tracing the contamination back to its source. A fast, specific and sensitive *in vitro* method would also be of great help in securing microbiological food safety. The goals of this project are to develop methods that could be used for the detection and enumeration of *C. botulinum* in food-, faecal- and patient samples, as well as to study how environmental factors affect the neurotoxin production.

Materials and methods: DNA was purified from overnight cultures using the EasyDNA kit (Invitrogen). For gram-positive strains 8 mg/ml of lysosyme and 40 U/ml of mutanolysine were added and the sample was incubated at 37AC for 30 min before the purification. Several different primer pairs targeting the *bont* genes of *C. botulinum* types A and E were evaluated regarding specificity, sensitivity and reproducibility. The specificity of the PCR assay was evaluated using DNA from 8 strains of *C. botulinum* type A, 16 strains of *C. botulinum* type E, 16 strains of *C. botulinum* types B and F, 16 strains of other *Clostridium* spp., and 26 non-*Clostridium* strains. Conventional PCR was carried out on a Gene Amp 9700 thermal cycler in a total volume of 25 ml. For type A the primers and protocol were modified from Takshi et al. (1996). For type E the PCR mixture contained 1X PCR Buffer, 0,2 mM of each dNTP 0,5 mM of each primer, 1,25 U Taq DNA Polymerase, and 5 ml of template solution. The amplification commenced with a denaturation step at 94AC for 7 min, followed by 40 cycles consisting of heat denaturation at 94AC for 40 s, primer annealing at 58AC for 40 s, and extension at 72AC for 40 s. Finally extension was performed at 72AC for 7 min to complete the synthesis of all strands. The products were visualized by agarose gel electrophoresis. DNA-sequencing of the type E amplicon was performed at the BM-unit, Lund University, according to the chain-termination method (Sanger

et al. 1977). Several different hybridisation probes were tested for the *bont* E gene and a real-time PCR assay, using the LightCycler™ instrument, was developed. The hybridisation probes consist of two parts; a donor probe labelled with fluorescein at the 3'-end and an acceptor probe labelled with LC Red640 (LC) at the 5'-end and 3'-hydroxy blocked with a phosphate. The PCR mixture contained 1X PCR Buffer, 4 mM MgCl₂, 0.2 mM of each dNTP, 0.3 mM of each primer, 0.3 mM of each probe, 1.25 U Tth DNA Polymerase, and 4 ml of template solution. The total volume added to each capillary was 20ml. The amplification protocol used started with an initial denaturation at 95AC for 60 s, followed by 45 cycles of 95AC for 0s, annealing and fluorescence acquisition at 56AC for 5 s, and elongation at 72AC for 25 s. Primers and probes have also been developed for the *rrn* gene, a housekeeping gene, to allow relative quantification of gene expression. A modified method for total RNA extraction from *Bacillus* spp. with acidic phenol was used (Putzer et al., 1992). First-strand cDNA was synthesized in two separate RT assays using the reverse primers for the *bont* E gene and the *rrn* gene. cDNA synthesis was performed in a Gene Amp 9700 thermal cycler. The total volume of the reaction mixture was 20 µl and contained 0.1 µg total RNA, 0.5 mM of each primer, 5 mM of each nucleotide dATP, dTTP, dCTP and dGTP, 20 U RNasin® ribonuclease inhibitor, 5 mM DTT, 1× first-strand buffer and 200 U Superscript™ II RNase H⁻ reverse transcriptase. Before RT enzymes were added, the reaction mixture was heated to 65°C for 5 min and thereafter chilled on ice. After brief centrifugation and the addition of RT enzymes, the reaction mixture was incubated at 42°C for 50 min and the reaction was terminated by incubation at 70°C for 15 min. The real-time PCR protocol described above was used, but using 0.5 mM of the primers for the *rrn* gene.

Results and discussion: All 8 strains of *C. botulinum* type A could be detected using the conventional PCR, while none of the other strains gave any bands. The detection limit was at least 0.5 ng/ml, corresponding to 2.5 pg of total DNA in the tube. During the comparison of primers and probes some anomalies were detected among the type E strains. Therefore the amplicons were sequenced. A difference from the sequences reported in GenBank was found in two of the strains. All of the tested *C. botulinum* type E strains could be detected using the method and none of the other strains gave any detection. The detection limit for the real-time assay was at least 0.1 ng/ml, which corresponds to 0.4 pg of total DNA in the capillary. When following the relative expression of two different strains of *C. botulinum* type E it was found that the expression of the neurotoxin was dependent on when in the growth cycle the sample was taken. Preliminary results show that the neurotoxin gene expression increases during the exponential phase, reaching its maximum as the bacteria enter in the stationary growth phase. This agrees with previous findings for other *C. botulinum* type E strains [McGrath, 2000], as well as with *C. botulinum* type B (Lövenklev et al. manuscript).

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SAMPLING CECAL CONTENTS OR ILEOCECAL LYMPH NODES: IS IT DIFFERENT?

O 30

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Summary: The objective of this study was to compare the prevalence of *Salmonella enterica* in swine populations estimated by sampling cecal contents versus ileocecal lymph nodes. In each of two abattoirs, four groups of pigs (n=30 pigs per group) were studied. Cecal contents and ileocecal lymph nodes were individually collected and processed for isolation and identification of *S. enterica*. The overall prevalence found by cecal contents was 40%, whereas by ileocecal lymph nodes it was 22.9% ($p<0.05$). Combining results from both samples, the prevalence found was 50.8%. The relative sensitivity of cecal content sampling was 79.3%, whereas for ileocecal lymph node sampling it was 45.5%. The agreement (Kappa statistic) between both sample types was 13.1%. This study demonstrates that sampling either cecal contents or ileocecal lymph nodes affects results of *S. enterica* epidemiological studies. It is recommended that both samples be used.

Keywords: *Salmonella*, Swine, Prevalence, Sample types, Comparison

Introduction: When trying to understand the epidemiology of *Salmonella enterica* in the pork production chain, sampling represents an important variable to be critically considered. Samples commonly used for diagnosis of subclinical *Salmonella* infection in market weight pigs include rectal swabs, feces, lymph nodes, cecal contents, and rectal contents. Recent studies (Swanenburg et al.,2001; Hurd et al.,2002) reported variable results based on different sample types, indicating that differences in samples collected may affect results of descriptive and comparative studies. Not only it is common to find studies that compare different sample types (unmatched), but also, it is difficult to compare results between studies, as different sample types are frequently used. The objective of this study was to compare the prevalence of *Salmonella enterica* in swine populations estimated by sampling cecal contents versus ileocecal lymph nodes.

Materials and Methods: In each of two abattoirs, four groups of pigs were studied (150 pigs per group). From each studied group, 30 animals were randomly selected on the slaughter line. Cecal contents (10g) and ileocecal lymph nodes were individually collected and processed by standard bacteriological methods for isolation and identification of *S. enterica*, as previously described by Rostagno et al.(2003). Variables analyzed included: the proportion of positive samples by abattoir, by studied group, and by sample type. The data analysis included frequency distribution analysis for each variable, cross tabulations and comparison of proportions, using Chi-square test ($p<0.05$). The relative sensitivity for cecal content and ileocecal lymph node samples was estimated by comparing the proportion of pigs positive by each sample type to the proportion of pigs positive by at least one of the samples analyzed. The agreement between sample types was determined by the Kappa statistic.

Results: The overall *S. enterica* prevalence found by cecal contents was 40%, whereas by ileocecal lymph nodes it was 22.9% ($p<0.05$). The *S. enterica* prevalence in abattoir A, determined by cecal contents and ileocecal lymph nodes was 36.7% and 27.5% ($p<0.05$), respectively. In abattoir B, the prevalence found was 43.3% and 18.3% ($p<0.05$), respectively. Combining results from both samples, the prevalence found was 47.5% in abattoir A, 53.3% in abattoir B, and 50.8% overall. A total of 13 different *S. enterica* serovars was isolated (11 from cecal contents and 9 from ileocecal lymph nodes). Four serovars were isolated only from cecal contents (Montivideo, Ohio, Mbandaka and Bovis-

morbificans), whereas 2 serovars were only isolated from ileocecal lymph nodes (Choleraesuis var. kunzendorf and 4,12:autoagglutinable). *S. enterica* serovars isolated from both samples included; Typhimurium var. Copenhagen, Typhimurium, Derby, Anatum, Agona, Newport and Heidelberg. The relative sensitivity of cecal content sampling was 79.3%, whereas for ileocecal lymph node sampling it was only 45.5%. The agreement (Kappa statistic) between both sample types was only 13.1%.

Discussion: This study demonstrates that cecal contents and ileocecal lymph nodes constitute different sources of information on *S. enterica* epidemiology in swine populations. Their individual use may have impact on results reported. When sampling cecal contents, generally, a higher prevalence is expected, based on its higher sensitivity (79.3 versus 45.5%). However, even though cecal contents are more sensitive than ileocecal lymph nodes, some positive animals will be missed. The poor agreement found (13.1%) indicates that both samples should be considered for a better estimate of the *S. enterica* prevalence, as they are complementary samples. Our results are in agreement with Swanenburg et al.(2001) and Hurd et al.(2002), reporting difference in prevalence estimates in slaughter pigs, based on different sample types. We hypothesize that the invasiveness of a serovar and the period of time elapsed between exposure and sample collection constitute determinant factors for the prevalence and serovar diversity found in each sample type. Probably, ileocecal lymph nodes reflect on-farm infections and rapid infections acquired from pre-slaughter contaminated environments (transport trailers and abattoir holding pens) by more invasive serovars, whereas cecal contents may reflect both on-farm and rapid infections or contaminations of the gastrointestinal tract after pigs leave the farm.

Conclusions: This study demonstrates that sampling either cecal contents or ileocecal lymph nodes affects quantitative (prevalence), as well as qualitative (serovar diversity) results of *S. enterica* epidemiological studies. It is recommended that both samples be used to get a better epidemiological picture of *S. enterica* in swine populations.

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Proficiency Test of Four *Salmonella* Antibody ELISA-Tests for their Harmonization

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Keywords: ring test, test congruence, test sensitivity, herd categorization

Summary: The paper describes the necessity to only use tests for national salmonella monitoring and reduction programmes that are "harmonized", i.e. that produce at least at herd level the same results. Four in Germany licensed tests were audited in a proficiency test by four independent and neutral laboratories. The test was designed rather to harmonize the tests, if necessary, than to evaluate the single tests. The methods used to provide a high credibility for the proficiency test's outcome are explained. The conclusion of the ring test is that three of the four tests can be used for the salmonella monitoring programme in Germany as long as not single results are compared to each other, but the results of sets of sera are used for the herd categorization for their risk level of introducing *Salmonella* spec. into the food chain.

Introduction: In Germany, a national "Salmonella Monitoring and Reduction Programme" (Blaha, 2003) has been implemented, which started in 2002 has been mandatory since April 1, 2003, for those farmers and slaughter plants that participate in the German newly developed quality management system for food, the so-called "QS-System" (Anonymous, 2003). In the framework of its implementation, the following problem emerged: when the programme started, 4 *Salmonella*-antibody ELISA tests had been licensed by the veterinary authority of Germany. This means that it is the right of anybody participating in the programme to use any of the 4 licensed tests. However, when the tests were being licensed some time ago, there was no national programme and, thus, no need for making sure that testing sets of random samples of a herd tested with all four tests should produce the same results. Due to this fact, the producers of the four tests defined their test specific algorithms for identifying a certain OD% value independently of each other. The result is that there was the perception that the tests differ in their sensitivity. It could be predicted that an ongoing discussion about which results are right and which are wrong would be unpreventable, since farmers will start to claim that their herd could be negative if only another test had been used. There were two possibilities to handle this: a) the QS-System had to decide to accept only one of the tests, or b) the tests had to be "harmonized". Soon there was a consensus not to hinder the free competition between different test providers, i.e. a proficiency test had to be organized to objectively measure to which degree the tests really differ from each other.

Material and Methods:

Test design: Based on the experience of the first international ring trial of ELISAs for antibody detection in swine (van der Heijden, 2001), the test was planned and conducted under the leadership of the Field Station for Epidemiology of the School of Veterinary Medicine of Hanover, which also served as one of the four independent test laboratories that have no conflict of interest (the three other labs were: the Department of Bacterial Animal Diseases of the Federal Institute of Viral Animal Diseases in Jena, and the Diagnostic Department of the Chamber of Agriculture of Lower Saxony in Oldenburg

and the Institute of Animal Health of the Chamber of Rhineland-Westphalia in Muenster). Since the test was designed and planned in full agreement of the test producers, they were asked to test the set of test sera, but only with their own test. In other words, the four neutral laboratories tested the set of test sera with all four tests, the producers tested the set of test sera only with their own test. The sera were randomly numbered and the laboratories and the tests were anonymized. All test participants were provided with a standardized Excel-table to make the data handling for the statistical analysis of the test results easier.

Test sera: Out of several thousand sera, the test results from one of the four ELISA tests of which were already known, 400 sera (200 meat juices, 200 blood sera) were selected. To make sure that the majority of the test sera were sera with expected OD% values "around" the target cut-off value of 40 OD%, the following range of values was chosen for the set of test sera for both the meat juice and the blood sera: 20% of the test sera with very low expected OD% values (0% or very close to 0%), 20% of the test sera with very high expected OD% values (above 100%), and 60% of the test sera with an expected OD% value around the cut-off value of 40%.

These sera were split into 8 aliquots and send to the four test laboratories asking them for "running the tests following the instructions as given by the test producers"- the four test producers just ran their own test without any particular instruction.

Data handling: 1) The four data sets of the producers were combined to one data set with all four tests so that, additionally to the four data sets of the neutral test labs (with four test results for every serum), there was a fifth data set with all four test results for every serum: the "producers' data set". This resulted in 5 data sets with results from 5 test participants having tested the same set of sera with 4 ELISA tests. 2) Although all test participants had gotten the same table to fill in, the data still had to be cleaned and tested for their plausibility, since some data were rounded up, others with a comma and some with dots; some high values were capped at a certain value, others were not etc.

Statistical analysis: Any statistical calculation was done using the SAS version 8.1e. 1) The calculation of mean and median values was performed traditionally. 2) The evaluation of correlations (two tests each) was estimated by scatter blots. 3) Box blots were constructed following the classical proposal of TUKEY (box range is from the 25 to the 75% quantile). 4) The variance analysis was performed (ANOVA) both on the original and the logarithmic scale. The comparative sensitivity analysis for the four tests was conducted by omitting the laboratories one by one and the ELISA tests one by one. 5) Finally, a descriptive compliance analysis was conducted by calculating the degree of congruence between the labs and the tests at varying cut-off values resulting in a variety of surface plots.

Results: All statistical testing of the four tests showed that the variance between the four tests was significantly higher than the variance between the five test laboratories. None of the tests correlated with one of the others completely, but one was clearly out of the range of the three other tests. The producer of this test agreed to retract it from the market until harmonized with the other three tests, although this test, as proven in the dossier for the licensing procedure, reliably recognizes sera with low and sera with high concentrations of *Salmonella* antibodies. The remaining tests were statistically tested again using the above-mentioned methods. This time, there was still a slight higher variance between the tests than between the laboratories (ring test participants), but this difference was not statistically significant.

Conclusion: These results suggest that the three remaining tests (produced by Labordiagnostik Leipzig, IDEXX, and Boehringer/Ingelheim) could be used alternatively for the *Salmonella* monitoring programme in the framework of the German "QS-System". However, this conclusion does not apply to the comparison of "positive/negative" results of single sera, since those sera with an antibody concentration around the cut-off value can to up to 20% be identified differently as positive or negative if different tests are used. However, if the result of sets of 60 (= minimum random sample per herd) are used for categorizing the corresponding herd as "low", "medium" or "high" risk herds, the inter-test variance of the tests is then definitively lower than the inter-laboratory variance of single

test results. In other words, although single sera, which have *Salmonella* antibody concentrations around the cut-off point, can be recognized as positive with one test and negative with the other test or vice versa, the three tests can be used, since less than 10% of the sera tested over years in Germany, have *Salmonella* antibody concentrations around the cut-off point (around 70% have very low titers, and about 15% have quite high titers, which are always correctly identified by all three tests as negative or positive). This means that the risk that a set of sera is categorized differently by using another test out of the three tests is so low that it can be neglected in the light of the overall goal of the German Salmonella Monitoring and Reduction Programme: Identifying via an ongoing semi-quantitative estimation the herds with the relatively highest risk of introducing zoonotic *Salmonella* spec. into the food production chain to be able to implement measures for a) reducing the cross-contamination of *Salmonella* spec. in the slaughter plant and b) reducing the salmonella load of swine herds identified as high risk herds. Finding the herds with the relatively highest salmonella risk, at which cut-off point ever, will be performed by all three tests at a comparative herd sensitivity level.

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ABBREVIATED IDENTIFICATION SCHEME FOR *ESCHERICHIA COLI* IN SWINE FECES

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Summary: Antimicrobial susceptibility profiles of *Escherichia coli* (EC) are often used to monitor the effect of antimicrobial use regimens on the antimicrobial resistance (AR) reservoir in animal species. Epidemiological studies of AR may involve the identification of thousands of bacterial isolates, so complete biochemical identification of EC can be prohibitively expensive and time consuming. In this study an abbreviated biochemical scheme using colony phenotype and the indole test results in a sensitivity and specificity of 91.7% and 100% respectively for identification of EC as compared to a commercial biochemical identification kit. This abbreviated scheme results in over US\$500 savings per 100 candidate EC isolates identified. These savings have significant benefits to the economics of conducting epidemiologic investigations of AR.

Keywords: Indole, Antimicrobial Resistance, API 20E, Sensitivity, Specificity

Introduction: The gram negative fecal flora represents a reservoir of antimicrobial resistance (AR) genes transferable to foodborne pathogens. *Escherichia coli* (EC) are often used to define the reservoir of AR. Epidemiological studies may involve testing thousands of isolates, so complete biochemical identification of EC can be prohibitively expensive. The goal of this project was to develop an inexpensive and effective method of identification of EC. Previous investigators (Hyatt et al., 2002) determined that 99.4% of colonies isolated from cattle feces that had typical EC phenotype on MacConkey agar and were positive for indole production were EC. According to the Manual of

Clinical Microbiology (Farmer, 1999), 95% of active EC ferment lactose and 98% of active EC are positive for indole production. Of inactive EC, 25% ferment lactose and 80% are indole positive.

Materials and Methods: In order to determine if the abbreviated protocol would be effective for swine feces we used the following methods. As part of a separate investigation (Funk et al., 2003) fecal samples from 154 randomly selected market age pigs representing 3 different farms were collected by a free catch method with a clean glove used for every sample. The samples were then placed in sterile Whirl-pak bags (Nasco, Ft. Atkinson, WI, USA) and transported to the lab. Samples were processed as previously described (Funk et al., 2003). One bacterial colony was selected from each sample for storage at -80°C for further AR testing. Stored isolates were streaked onto MacConkey agar (Becton Dickinson, Sparks, MD USA) and incubated at 37°C for 18-24 hours. Following incubation colonies were phenotypically compared to that of the control strains. Those colonies that were phenotypically consistent with EC were then inoculated into 4mL of DEV Tryptophan broth (EM Science, Gibbstown, NJ USA) for the detection of indole production. Following incubation at 37°C for 18-24 hours, 3-5 drops of Kovac's reagent was added to each tube. A red ring at the top of the tube represented the presence of indole. All indole positive isolates were identified as EC by the abbreviated method. All isolates were also inoculated onto an API 20E strip according to manufacturer's instructions (BioMeriux, Hazelwood, MI USA).

Results: Of the 154 isolates tested, 144 were identified as EC by the API 20E. The other 10 isolates were *Klebsiella* (2), *Salmonella* (2) or unidentifiable (6). One hundred thirty-two isolates were identified as EC by the abbreviated scheme. When the unidentified isolates were coded as non-EC using the gold standard, the sensitivity and specificity of the abbreviated scheme was 91.7% and 70.0% respectively (Table 1). The positive predictive value was 97.8%, and the negative predictive value was 37.0%. When isolates unidentifiable by the API 20E were not included in the analysis, the sensitivity remains 91.7% and the specificity improves to 100%. The positive predictive value becomes 100% and the negative predictive value becomes 25.0% (Table 2). As a consequence of the results of this study, our lab protocol is as follows: Isolates are struck onto MacConkey agar and incubated at 37°C for 18-24 hours, those isolates that phenotypically appear to be EC are then inoculated in 4mL of DEV Tryptophan broth and incubated at 37°C for 18-24 hours. Following incubation isolates were tested for the presence of indole, using Kovac's reagent. All isolates that are not identified as EC by the abbreviated scheme are identified using the API 20E strips.

Discussion: In epidemiological investigations of AR, there is a need to identify large numbers of bacteria. When looking at large numbers of samples there are often two limiting factors, time and finances. In adopting this method of identification a substantial amount of money can be saved with very little labor cost as compared to traditional biochemical identification systems. Using the gold standard method there would be a cost of US\$541.00 for every 100 isolates. Using the method described above there is a cost of US\$34.65 for every 100 isolates, assuming 95% of the isolates are EC. There is a savings of US\$506.35 for every 100 isolates. These savings have significant impact of the financial resources necessary for conducting epidemiological studies.

Table 1. Sensitivity and specificity of abbreviated scheme as compare to the API 20E for biochemical identification of *E. coli*. Isolates unidentified by API are coded as non-*E. coli*.

| | | Gold Standard (API 20E) | | |
|---------------------------|----------|-------------------------------|--------------------------|-------------------|
| | | Positive | Negative | |
| <i>Abbreviated Scheme</i> | Positive | 132 | 3 | PPV=132/135=97.8% |
| | Negative | 12 | 7 | NPV=7/19=36.9% |
| | | Sensitivity=132/144 =91.7% | Specificity=7/10 =70% | |

Table 2. Sensitivity and specificity of abbreviated scheme as compare to the API 20E for biochemical identification of *E. coli*. Isolates unidentified by API are not included in analysis.

| | | Gold Standard (API 20E) | | |
|---------------------------|----------|-------------------------------|--------------------------|------------------|
| | | Positive | Negative | |
| <i>Abbreviated Scheme</i> | Positive | 132 | 0 | PPV=132/132=100% |
| | Negative | 12 | 4 | NPV=7/19=25% |
| | | Sensitivity=132/144 =91.7% | Specificity=4/4 =100% | |

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Isolation of *Salmonella enterica* in seropositive classified finishing pig herds

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Summary: The aim of this study was to assess the probability of detecting *Salmonella* from pen faecal samples in seropositive classified finishing pig herds. The study involved 77 herds from Denmark (20), the Netherlands (20), Greece (17) and Germany (20). The serological herd status was determined by the blood- sampling of 50 finishing pigs. Bacteriological sampling was performed by 20 pen faecal samples per herd. Over-all, 47 % of the blood samples had an OD% larger than 10 and 23 % larger than 40. *Salmonella* was isolated from 135 (9.3 %) pen faecal samples in 32 herds (42 %). Twenty-eight of these herds (87.5 %) had a within-herd seroprevalence larger than 50% at sample cut-off OD%>10. A correlation coefficient of 0.62 was found between the proportion of culture positive- and seropositive samples in a herd at cut-off OD % > 10 and of 0.58 at cut-off OD % > 40. Due to the low sensitivity of culture methods, apparent 'false positive' serological results may well represent real infections not detected by bacteriological testing. In this study, there was an increasing probability of recovering *Salmonella* with increasing within-herd seroprevalence.

Keywords: pig-bacteria, herd-status, serology, bacteriology, epidemiology,

Introduction: Determining the *Salmonella*-status of pig herds as part of a monitoring and intervention programme to reduce the contamination of pork is necessary to direct interventions at high prevalence herds. In addition, slaughtering these herds separately from others will minimise cross-contamination during the harvest-phase (Mousing et al, 1997). Culturing faecal samples for *Salmonella* is a useful tool to determine current infections in a pig herd. However, conventional culture methods are labour intensive, time consuming and expensive and may therefore not be practical or economically feasible for routine application. Modern serological techniques have proven to be convenient and cost effective methods for screening for antibodies against *Salmonella*, indicating exposure in pigs (Mousing et al., 1997). To assess the probability of detecting *Salmonella* in seropositive classified pig herds, pen faecal sampling was performed in herds with at least one serological sample over 10 OD %. The investigation was part of an international research programme, entitled "*Salmonella* in Pork" (FAIR1 CT95-0400) or SALINPORK (Lo Fo Wong and Hald, 2000). This study was performed between August 1996 and July 1998 and involved herds from Germany, Denmark, Greece and the Netherlands.

Materials and Methods: A total of 77 pig herds participated from Denmark (n = 20), the Netherlands (n = 20), Greece (n = 17) and Germany (n = 20). Danish and Dutch herds were selected, based on their high seropositive status, while German and Greek herds were not. Due to these selection procedures and small numbers, these herds are not representative for the actual prevalence in these countries. The serological *Salmonella* status of each herd was assessed in a related investigation (Lo Fo Wong and Hald, 2000) by testing 50 blood samples from market weight pigs. Blood samples were analysed with an indirect mix-ELISA (Nielsen et al., 1995; van der Heijden et al., 1998). Samples with an Optical Density Percentage (OD %) larger than 10 were considered seropositive. After the serological status of a herd was assessed, the herd was re-visited and 20 pooled pen faecal samples, of i.e. 5 times 5 grams each, were collected randomly from pens with fattening pigs. Spearman's rank correlation test was used to calculate non-parametric correlation coefficients. The association between the proportion of *Salmonella* isolates from a herd and the within-herd seroprevalence was modelled by linear logistic regression (PROC GENMOD, SAS Institute, 1996) and adjusted for between-country variation and herd-level clustering.

Results: A total of 4194 blood samples were collected in 77 herds, of which 1977 (47 %) had an OD % larger than 10 and 985 (23 %) larger than 40. *Salmonella* could be isolated from 135 (9 %) out of a total of 1455 pen faecal samples. These positive samples originated from 32 (42 %) herds. The bacteriological results in this study are presented in detail in Table 1. Over-all, a correlation coefficient of 0.62 ($p < 0.0001$, Spearman) was found between the proportion of seropositive samples and the proportion of culture positive samples in a herd at cut-off OD % > 10, and of 0.58 ($p < 0.0001$, Spearman) at cut-off OD % > 40. The correlation between serological and bacteriological results is illustrated in Figure 1. In high seroprevalent herds, the recovery rate was markedly higher.

Table 1. The proportion positive herds and samples in Germany, Greece, Denmark and the Netherlands and the serotypes isolated from pen faecal samples.

| | Germany ¹ no. herds (%) | Greece ¹ no. herds (%) | Denmark ² no. herds (%) | Netherlands ² no. herds (%) |
|------------------------|---------------------------------------|--|---------------------------------------|---|
| Prop. positive herds | 1/20 (5.0 %) | 4/17 (23.5 %) | 17/20 (85.0 %) | 10/20 (50.0 %) |
| Prop. positive samples | 5/303 (1.7 %) | 4/340 (1.2 %) | 95/400 (23.8 %) | 31/412 (7.5 %) |
| Serotypes (serogroup) | 5 Derby (B) | 2 Typhimurium (B) 1 London (E1) 1 Bredeney (B) | 95 Typhimurium (B) | 20 Typhimurium (B) 9 London (E1) 1 Bovismorbificans (C2) 1 I, O:21, nm (L) |

¹ herds randomly selected; ² herds selected based on high seroprevalence

The regression equation for the predicted means was:

$$\text{logit}(y/n) = -4.35 + 3.53 * P_{\text{finishers}}$$

where: y = no. of isolates; n = no. of pen samples; and, $P_{\text{finishers}}$ = prop. seropositive finishers.

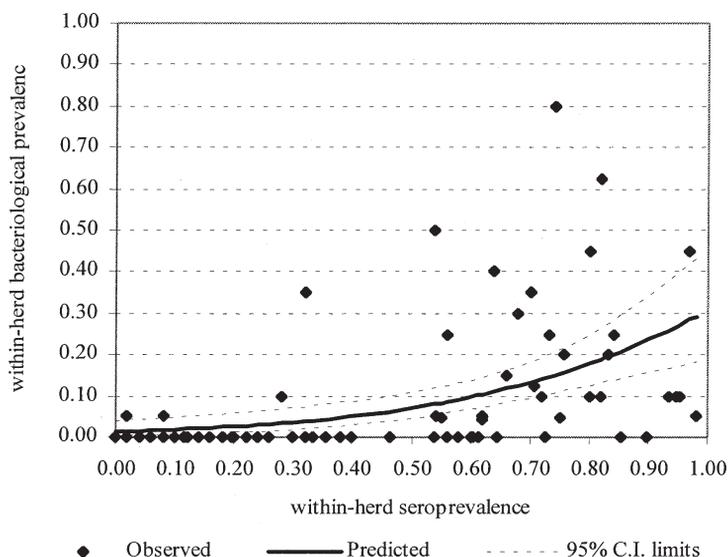


Figure 1. The correlation between the within-herd seroprevalence and the within-herd bacteriological prevalence, the curve of predicted values and 95% confidence limits, based on samples from The Netherlands, Germany, Denmark and Greece.

Discussion: There was only a moderate correlation between herd serological and bacteriological results in this study. *Salmonella* could not be isolated in a number of seropositive herds. These apparent 'false positive' results can either be caused by cross-reactivity, a low test cut-off value or by false negative reactions in the reference test. The sensitivity of culture methods is generally low. The presence of latent carriers or intermittent shedders in a herd may decrease the sensitivity of the bacteriological sampling method even further. In contrast to culture methods, latent carrier pigs can be identified through the detection of antibodies against *Salmonella*, provided the O-antigens of the serovar are included in the test. Therefore, a 'false positive' result could represent a real infection which was not detected by bacteriological sampling. Serology is a measure of historical exposure, which may or may not correlate closely to the microbiological burden at the time of sampling. However, for screening purposes, serological testing provides an indication of exposure to *Salmonella*, which forms the basis for targeted sampling, intervention and logistic slaughter procedures. In Denmark, serological screening followed by bacteriological follow-up has proven to be a successful approach in the National *Salmonella* Surveillance and Control Programme.

Conclusions: There is an increasing probability of recovering *Salmonella* with increasing seropositivity in the mix-ELISA.

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Pork and the number of human multi-resistant *Salmonella* Typhimurium DT104 cases

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Summary: As part of a revision of the Danish *Salmonella* policy, we estimated the impact of nationally produced pork compared to imported pork on the number of human sporadic domestic cases of multi-resistant *Salmonella* Typhimurium DT104 (MRDT104) in Denmark. We also estimated the number of deaths related to the presumed excess mortality associated with MRDT104. Data on exposure from domestic and imported pork were built into a simple simulation model in @Risk, and Monte Carlo simulations were used. Our results showed that imported pork resulted in 20 times as many human cases as domestic (2 human cases per year), and 1 extra death in 50 years. If the prevalence of MRDT104 in domestic pork increased 5 times, the absolute number of human cases (related to Danish pork) would be 8-11. The excess mortality due to this rise in human cases will be negligible compared to the mortality caused by other Salmonellae.

Keywords: food safety, human health, risk assessment, surveillance, trade

Introduction: Multi-resistant *Salmonella* Typhimurium DT104 (MRDT104) is of primary interest in many countries because of concern for human health. It has been suggested, that MRDT104 is associated with an excess mortality among humans. In Denmark, an eradication policy was initially carried into effect in the swine sector. This policy, among others, included depopulation of affected swineherds and mandatory bacteriological follow-up in herds with high levels of antibodies against *Salmonella*. MRDT104 spread despite the extensive means taken.

As part of a revision of the Danish *Salmonella* policy, we were interested in estimating the impact of nationally produced pork compared to imported pork on the number of human sporadic domestic cases of MRDT104 occurring in Denmark. We also wanted to estimate the number of deaths to expect because of the presumed excess mortality associated with MRDT104.

Materials and Methods: Data on exposure from domestic and imported pork were compared. Exposure was measured as the product between the relative amounts of pork consumed and the prevalence of MRDT104 in domestic and imported pork, respectively. Data describing *Salmonella* prevalence, the prevalence of MRDT104, and the number of human cases covered the time period 1998 to 2002. A simple simulation model was built in @Risk, and Monte Carlo simulation with 10,000 simulations was used. Pert distributions (with minimum, mode, and maximum) were used for all input parameters (Table 1).

Table 1. Description of variables in simulation model for the assessment of the impact of domestic and imported pork on the number of human MRDT104 cases in Denmark

| Variable | Minimum | Mode | Maximum | Data source |
|---|---------|---------|---------|---|
| Part of pork consumed in Denmark of imported origin | 7% | 10% | 12% | Danish Bacon & Meat Council, Danmarks Statistik |
| Part of pork consumed in Denmark of domestic origin | 88% | 90% | 93% | Danish Bacon & Meat Council, Danmarks Statistik |
| MRDT104 prevalence in imported pork | 0.4% | 1.3% | 1.7% | Danish Veterinary & Food Administration |
| MRDT104 prevalence in domestic pork | 0.005% | 0.0065% | 0.01% | Danish Bacon & Meat Council |

The number of human deaths due to the presumed excess mortality was estimated based on published results from Helms et al. (2002), as modified by Dahl (2003). These results showed, that the excess risk of dying was nearly significant ($p=0.06$) for cases caused by penta-resistant MRDT104, when including quinolone-resistant isolates, compared to cases caused by susceptible isolates (neither penta-, nor quinolone-resistant) (Table 2). When cases caused by quinolone-resistant strains were excluded, there was no significant excess mortality for penta-resistant MRDT104, compared to susceptible isolates ($p=0.55$). Assuming, that penta-resistant MRDT104 causes an excess mortality, even though it is not significant, we can estimate the excess number of human deaths caused by penta-resistant MRDT104, excluding the effect of background mortality and the mortality due to salmonellosis in general. Helms found, that the background mortality in the human control population was 1.1 % for 2 years. Using the relative risk (RR) estimates from Table 2 gives a mortality in the susceptible group of $1.1\% \times 2.1 = 2.3\%$. In the penta-resistant, quinolone-susceptible group this figure is $1.1\% \times 2.9 = 3.2\%$. The excess mortality due to penta-resistance is then $3.2\% - 2.3\% = 0.9\%$.

Table 2. Two-year relative death risks of 2,047 Danish patients infected with *Salmonella* Typhimurium, by antimicrobial susceptibility pattern. 1995-1999 (Helms et al, 2002, modified by Dahl, 2003)

| Resistance pattern of isolates | Deaths/cases | RR ^a (C. I.) | P-value ^b |
|-----------------------------------|--------------|-------------------------|----------------------|
| Penta including quinolone | 12/283 | 4.8 (2.2-10.5) | 0.06 |
| Penta with quinolone | 5/40 | 13.1 (3.3-51.9) | 0.01 |
| Penta without quinolone | 7/243 | 2.9 (1.1-7.9) | 0.55 |
| Susceptible <i>S. Typhimurium</i> | 47/1764 | 2.1 (1.5-2.9) | 1.00 |

^aDeath risk relative to the general population (random matched sample of 20,456 Danes), adjusted for co-morbidity (Helms et al. 2002). ^bComparison of RR with susceptible *S. Typhimurium* isolates (last row)

Results: According to the model, imported pork resulted in 20 times as many human cases as domestic. The model was validated against the Danish Zoonosis Centre's model, which produced similar results. By use of the ratio between MRDT104 and *Salmonella* spp. identified in pork in Denmark, it was estimated, that Danish pork was responsible for 1.6-2.2 (M2) human domestic cases of MRDT104 per year. If the prevalence of MRDT104 in domestic pork increased 5 times, the absolute number of human cases (related to Danish pork) would be between 8 and 11 cases. The 2 human domestic cases both have an excess mortality of 1 % caused by MRDT104. This means that MRDT104 in Danish pork causes one extra death in 50 years at the present level.

Discussion: The Danish swine industry has invested a total of 20 million Euro – hereof 4 millions in year 2002 – on surveillance and control of MRDT104. This amount should be seen in relation to the 8 million Euros spent annually on *Salmonella* reduction in pigs and pork. The industry has committed itself to reducing the prevalence of *Salmonella* by 27.5 % in the next 5 years.

A lift on the strict regulation on MRDT104 will most likely results in an increase in the number of human cases. Such an increase will to some extent be counteracted by the industry's general effort against *Salmonella*. Even with a substantial increase in the MRDT104 prevalence in the pig production, the excess mortality due to this source will be negligible, when the total number of human *Salmonella* cases ascribed to pork is as low as it is in Denmark (163 reported cases in 2001, 77 in 2002).

Conclusion: This study demonstrates the importance of focusing on the absolute size of a problem in order to allocate resources most cost-effectively in a national surveillance-and-control programme.

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Estimated society cost for pork-related *Salmonella* and *Yersinia* in Denmark 2002

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Summary: The Danish society costs for human gastroenteritis from pork related *Salmonella* and *Yersinia* have been estimated for 2002. The expenses for the veterinary salmonella- program in 2002 are calculated to 6.9 million Euro. The registered human cases only represent the more severely affected human cases; the total number of human cases might be 5 to 20 times higher. The registered salmonellosis and yersiniosis cases are assumed to have lost 8 and 7 working days through sickness; whereas the not registered cases are assumed to have lost 2 working days, respectively. The Danish society costs in 2002 for pork related salmonellosis and yersiniosis due to gastroenteritis are estimated to 0.16 – 0.59 million Euro and 0.32 – 1.3 million Euro, respectively. Between 70-90% of the society expenses are due to lost days of work.

Keywords: zoonoses, swine, lost days of work, public health economy

Materials and methods: The aim of the study is to estimate the Danish society costs for human gastroenteritis from pork related *Salmonella* and *Yersinia* in 2002, the effect of the control programs on the number of infected Danes and the amount of saved society expenses from 1994 to 2002. Sequelae, chronic health effects and premature death are not included in the estimates. A national *Salmonella* surveillance and control program for swine have been implemented in Denmark since 1995 (Nielsen et al. 2001). The level of *Salmonella* is controlled at various stages: feedstuffs, breeder and multiplier herds, weaner-producers, finisher herds and at the slaughterhouse. The registered human incidence of pork related salmonellosis has declined from 22 per 100.000 inhabitants in 1993 to 1.4 per 100.000 in 2002 (Anonymous 2003). The expenses for the veterinary salmonella-program in 2002 were calculated to 6.9 million Euro, of which the swine industry covers 92%. No specific veterinary *Yersinia* control program exists in Denmark. However, slaughter hygiene has steadily been improved during the last decade and is considered to have reduced the prevalence of *Yersinia* in pork. The registered human incidence of Yersiniosis has declined from 14 per 100.000 inhabitants in 1993 to 3.8 per 100.000 in 2002 (Anonymous 2003). There is no source account for human yersiniosis in Denmark, but we assume that at least 80% of the Yersiniosis cases are food borne (Mead et al 1999), and that all originate from Danish pork. However, the registered human cases only represent the

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more severely affected human cases, and the total number of human cases might be 5 to 20 times higher. The presented estimates include registered cases that are: a) hospitalised; b) hospitalised and undergoing surgery; c) tested positive by a general physician as well as infected but not registered cases where: d) no positive faecal sample is taken by physician; e) physician is not consulted.

The society expenses for consulting a Danish physician in general practise were 10 Euro, and the culture expenses for *Salmonella* and *Yersinia* were in average 120 Euro and 131 Euro, respectively. In Denmark, 26.5% and 11.8% of the registered salmonellosis and yersiniosis cases are hospitalised. Of the hospitalised cases 2.3% and 1.8% undergo surgery (Helms, personal communication). A patient submitted to hospital with gastroenteritis in 2002 is estimated to cost 2.810 Euro and cases undergoing surgery cost 8.958 Euro. Registered salmonellosis cases are assumed to have 13 days of sickness (Mølbak et al, 2003), whereas the registered Yersiniosis cases have 11.5 days of sickness (Germer-Smidt, personal communication). The number of working days in Denmark is on average 221 per year (61% of all days), and if the case is a child, a parent is assumed to have lost days of work. An average lost working day, estimated as 75% of the average wages for daya public employee in 2002, is estimated to 160 Euro.

Results: The estimated society expenses, medical and lost working days, for human cases of salmonellosis and yersiniosis are shown in table 1.

Table 1. Estimated society expenses, medical and lost working days, for human cases of salmonellosis and yersiniosis in Denmark 2002.

| Level | Lost working days, Salmonella | Expenses Euro | Lost working days, Yersinia | Expenses Euro |
|-------------------------|----------------------------------|------------------|--------------------------------|------------------|
| a) Hosp. + Surgery | 8 | 10,200 | 7 | 10,10 |
| b) Hospitalised | 8 | 4,000 | 7 | 3,900 |
| c) Physician, + culture | 8 | 1,400 | 7 | 1,300 |
| d) Physician, - culture | 2 | 300 | 2 | 300 |
| e) At home | 2 | 300 | 2 | 300 |

In 2002, Danish pork is assumed to be the source of 77 registered cases, equivalent to a cost of 165,300 Euro. Table 2 shows the estimated number of cases and annual expenses for the registered salmonellosis cases; as well as for non-registered cases assuming only 5% of the "real" infected cases are registered.

Table 2. The estimated annual expenses 2002 for the salmonellosis cases, where only 5% of the "real" infected cases are registered. All expenses are in Euro. * Assume 35% consult a physician.

| Level | % of cases | Estimated No. of cases | Exepenses, society total | % Expenses, Lost work |
|----------------------------|-------------|---------------------------|-----------------------------|-----------------------------|
| Reg cases: | | | | |
| a) Hosp. + surgery | 0.031% | 0-1 | 4,800 | 12% |
| b) Hospitalised | 1.3% | 20 | 81,300 | 31% |
| c) Physician, + culture | 3.7% | 57 | 79,000 | 91% |
| Unregistered cases: | | | | |
| d) Physicial, - culture | 30% | 462 | 139,400 | 97% |
| e) At home* | 65% | 1,001 | 292,600 | 100% |
| Total | 100% | 1,540 | 601.100 | 88% |

Table 3 summarizes the expenses, number of registered case and estimated "real" cases, when 5% to 20% of the cases are registered.

Table 3. The Danish society costs in 2002, human medicine and lost days of work, for pork related salmonellosis and yersiniosis assuming 5-20% of the real cases are registered.

| Infection | Reg. cases | Estimated no. of "real" cases | Estimated society expenses for "real" cases |
|---------------|------------|-------------------------------|---|
| Salmonellosis | 77 | 385 – 1,540 | 165,300 – 601.100 |
| Yersiniosis | 192 | 960 – 3,840 | 324,300 – 1.392.000 |

Discussion: The veterinary *Salmonella* control program for swine have reduced the pork related number of human salmonellosis cases from 1,144 cases registered in 1993 to 77 cases in 2002. The annual expenses to the program were 6.9 million Euro in 2002. In total, the expenses to the veterinary program have been close to 90 million Euro since its initiation in 1995. Assuming that the number of human cases had remained at the pre-control level if the salmonella-program have not been implemented, the program have reduced the occurrence of human salmonellosis in Denmark by approximately 1,000 registered cases in 2002. The majority of human salmonellosis cases are not registered, as only the more severely affected patients are examined by culture, so the "real" number of salmonellosis cases avoided in 2002 was probably 5,000-20,000, equivalent to a saved society cost of at least 3.5-8.2 million Euro. Between 70–90% of the society cost to human salmonellosis and yersiniosis is due to lost working days. From a society point of view, the salmonella-program and the improved hygiene on slaughterhouses have significantly reduced the *Salmonella* and *Yersinia* related expenses to hospitalisation, consultation to physicians and lost days of work.

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Comparison of *Campylobacter coli* strains isolated from pigs and humans - porcine strains a possible source of human infection?

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Summary: The primary aim of this study was to detect and genotype *Campylobacter* strains from pigs and humans. AFLP (amplified fragment length polymorphism) analysis was used to compare

different genotypes to identify the genetic diversity of *Campylobacter coli* (*C. coli*) strains. Heterogeneous patterns were detectable among the porcine and human *C. coli* pool.

By using an optimized extraction method combined with a PCR it was possible to detect *C. coli* DNA in some samples of the investigated minced meat but it could not be distinguished between dead bacterial cells and viable but nonculturable cell (VBNC)-forms of *C. coli* strains.

Keywords: AFLP; minced meat; paramagnetic beads; PCR; VBNC

Introduction: *Campylobacter* spp. are one of the very common causes of infectious gastroenteritis in humans. In this respect *C. jejuni* is the most important species. But there is no sufficient information about the relevance and the source of human *C. coli* infection. *C. coli* is a commensal of the gut of pigs and is excreted in faeces.

1. In order to assess the importance of pork as a source of *C. coli*-induced campylobacteriosis in humans it was necessary to detect and evaluate the prevalences of *C. coli* in pigs and in humans.

2. To characterize the genetic relationship of human and porcine *C. coli* strains, the genotypes of these were compared.

3. The consumption of minced meat may be a possible source for human infection with *C. coli*. Therefore we investigated different minced meat products bacteriologically and also by two different molecular biological methods.

Materials and Methods: For isolation of *C. coli* in fattening pigs (n=1150) a rectal swab was placed directly into Bolton-Broth and then cultured on mCCDA. The enrichment broth and the plates were incubated microaerobically for 48 hrs at 42 °C.

After the bacteriological isolation of *C. coli* strains, the DNA was extracted using a DNA extraction kit and identified using the PCR-technique (Linton, Lawson, et al. 1997 125 /id). Specific primers for the genus *Campylobacter* and the species *C. coli* were used.

For investigation of human *C. coli*, isolates were submitted to use from two regional laboratories as "thermophilic campylobacters" (n=456). Up to three single colonies from each agar plate were further processed by DNA extraction and PCR-technique described above.

In this study, AFLP was used for genetic typing of *C. coli* strains from pigs and humans as described previously (Duim et al., 1999).

To detect *C. coli* in pork products, minced meat was investigated bacteriologically and by PCR analysis. In addition an optimized PCR-technique was developed for a more sensitive detection of *C. coli* in minced meat. Boiled meat samples were mixed with a *C. coli* specific DNA-probe. After hybridisation paramagnetic beads were added to the mixture and incubated for 30 min at room temperature. *C. coli* specific PCR followed the extraction procedure.

Results: *C. coli* were detected in approximately 90 % of the fattening pigs in faeces (n=1150) and 21.5 % of human campylobacter cases bacteriologically examined (n=456). In the slaughter house we isolated *C. coli* at 85 % in faeces, 20 % on skin surfaces before chilling. After the chilling process no *C. coli* could be detected on the skin surfaces bacteriologically.

Human *C. coli* strains showed like porcine *C. coli* strains heterogeneous AFLP patterns. Nonetheless single similarities between human and porcine strains (isolated in the same region at similar times) could be established.

Culturable thermophilic *Campylobacter* spp. could not be detected in minced meat (n=125) bacteriologically. By direct PCR, the detection rate of *C. coli* increased slightly to 0.8 %.

When using an optimized extraction method combined with a PCR it was possible to detect *C. coli* DNA in higher numbers of the investigated minced meat samples.

Discussion: *C. coli* infection in humans is underestimated. At least 20 % of human campylobacteriosis are caused by *C. coli*. But the sources of *C. coli* infection in humans are not known.

The chilling process of pig carcasses in slaughter houses reduced the rate of culturable campylobacters on the carcass surface significantly.

Genotyping of *C. coli* revealed heterogeneous patterns among the human and porcine *C. coli* pool. It shows that different sources of infection in humans are most probable.

In minced meat we did not detect *C. coli* bacteriologically, but by the use of paramagnetic beads combined with PCR-technique we detected *C. coli* positive samples. This shows, that the meat was contaminated with *C. coli*. It is not clear what importance the presence of *C. coli* DNA in minced meat has for human infection, even though the presence of viable and culturable *C. coli* cells could be ruled out by bacteriological investigation. We could not distinguish between dead cells and VBNC forms of *C. coli* cells. The role of VBNC form of *C. coli*, a specific phenomenon of campylobacters (Lazaro et al., 1999) and certain other bacteria, has to be investigated further.

Conclusion: Porcine strains as sources of human *C. coli* infection can not be ruled out. Further research is needed to evaluate the *C. coli* findings in minced meat and the role of VBNC for human infection.

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Pathogenic bacteria and indicator organisms for anti-microbial resistance in pork meat at retail level in The Netherlands.

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Summary: Slaughter pigs and pork carcasses are often contaminated with pathogenic bacteria. Consequently raw meat on sale in retail stores may also contain these bacteria. In The Netherlands the calculated contribution by pigs to the relative occurrence of human salmonellosis in the period 1994-1998 was 25.2 % (van Pelt, 2001). Survey and monitoring data on the contamination of raw products with pathogens like *Salmonella*, *Campylobacter*, *Listeria monocytogenes* and *Escherichia coli* O157 are essential for making risk estimates, and the results of surveys carried out in 1990/2000 and 2002 are presented here. In 2002 also a surveillance of anti-microbial resistance among indicator bacteria (*Escherichia coli*, *Enterococcus faecium/faecalis*) isolated from pork meat was started. The results show that pork meat was contaminated with *Salmonella* in levels between 6.2 - 10.5 %, *S. Typhimurium* being the predominant serotype, and to a lesser extent with *Campylobacter*, *Listeria* and *E. coli* O157.

Keywords: *Salmonella*; *Campylobacter*; *Listeria monocytogenes*; *Escherichia coli* O157; *Enterococcus faecium/faecalis*.

Materials and methods: Raw meat products were sampled in the retail trade. Detection of *Salmonella*, *Campylobacter* and *Listeria monocytogenes* was carried out using standard methods. *E.coli* O157

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(VTEC) was determined using modified *E. coli* broth with novobiocin, immuno-magnetic separation and isolation on CT-SMAC agar. Isolation of the indicator organisms *E. coli* and enterococci was performed as follows. For *E. coli* 5 g of meat was incubated for 24 hours at 44 °C in 45 ml laurylsulphate broth, streaked on COLI-ID-plates and incubated 24 h at 44 °C, and identified by the AP80 test (Sensititre). Enterococci were isolated by incubation of 5 g meat in 45 ml azide dextrose broth at 44 °C for 24 h followed by streaking on Slanetz-Bartley medium and incubated for 48 h at 44 °C. Presumptive enterococcus isolates were identified by AP90 test (Sensititre). Susceptibility testing was done with Sensititre System (Trek Diagnostic Systems Ltd). Proportions of resistance were calculated based on breakpoints used in DANMAP and the NCCLS

Results: In the survey carried out in 1999/2000 *Salmonella* was found in 33 (6.2 %) of 533 samples pork meat, the predominant serotype being *S. Typhimurium* (14x) of which 3 isolates belonged to phage type D104. Other serotypes isolated were: Derby (2x), Brandenburg (2x), Bovismorbificans (2x), Enteritidis, Lancaster, Goldcoast, Livingstone, Infantis, Mbandaka, London, and *Salmonella* spp.(6x). *Campylobacter* was not isolated from 524 samples pork meat. VTEC O157 was detected in 1 (0.7 %) of 153 samples. In a more recent survey carried out in 2002, *Salmonella* was found in 11 (10.5 %) of 109 pork samples. Also here *S. Typhimurium* (4x) was the predominant serotype of which 1 isolate belonged to phage type D104. Also the serotypes Brandenburg, Infantis, London, Livingstone *Campylobacter* was isolated from 2 samples pork meat (2.1 %), VTEC O157 was not detected, and 2 samples contained more than 100 /g *Listeria monocytogenes*. For susceptibility testing 8 salmonellas, 53 strains of *E. coli*, 40 *E. faecalis* strains and 16 *E. faecium* strains were investigated. The results are shown in Table 1 and 2.

Table 1. Number of strains tested (N) and resistance proportions (R%) for *Salmonella* and *E. coli* from pork meat.

| Antibiotics | Breakpoint (µg/ml) | <i>Salmonella</i> | | <i>E. coli</i> | |
|----------------|--------------------|-------------------|------|----------------|------|
| | | N | R% | N | R% |
| Amoxicillin | >16 | 8 | 0 | 53 | 13.2 |
| Cefotaxime | >1 | 8 | 0 | 54 | 0.0 |
| Cefuroxim | >16 | 8 | 12.5 | 54 | 3.7 |
| Chloraphenicol | >16 | 8 | 0 | 54 | 0.0 |
| Ciprofloxacin | >2 | 8 | 0 | 54 | 0.0 |
| Doxycycline | >4 | 8 | 25 | 54 | 18.5 |
| Florfenicol | >16 | 8 | 0 | 54 | 0.0 |
| Flumequin | >4 | 8 | 0 | 54 | 0.0 |
| Gentamicin | >8 | 8 | 0 | 54 | 0.0 |
| Imipenem | >1 | 8 | 0 | 54 | 3.7 |
| Neomycin | >16 | 8 | 0 | 54 | 0.0 |
| Trimethoprim | >8 | 8 | 37.5 | 54 | 16.7 |
| Trim/sulpha | >8 | 8 | 37.5 | 54 | 13.0 |

Table 2. Number of strains tested (N) and resistance proportions (R%) for *E. faecalis* and *E. faecium* from pork meat.

| Antibiotics | Breakpoint (µg/ml) | <i>E. faecalis</i> | | <i>E. faecium</i> | |
|---------------------------|--------------------|--------------------|------|-------------------|------|
| | | N | R% | N | R% |
| Amoxicillin | >16 | 40 | 0.0 | 16 | 0.0 |
| Bacitracin | >128 | 40 | 15.0 | 16 | 6.3 |
| Ciprofloxacin | >8 | 40 | 0.0 | 16 | 0.0 |
| Doxycycline | >4 | 40 | 25.0 | 16 | 18.8 |
| Erythromycin | >4 | 40 | 15.0 | 16 | 25.0 |
| Flavomycin | >16 | 40 | 5.0 | 16 | 75.0 |
| Gentamycin | >500 | 40 | 7.5 | 16 | 0.0 |
| Lincomycin | >4 | 40 | 97.5 | 16 | 87.5 |
| Pirlimycin | >2 | 40 | 12.5 | 16 | 37.5 |
| Salinomycin | >16 | 40 | 7.5 | 16 | 6.3 |
| Strep > 1000 | >1000 | 40 | 12.5 | 16 | 6.3 |
| Strep > 2000 | >2000 | 40 | 2.5 | 16 | 0.0 |
| Quinupristin/dalfopristin | >4 | 40 | 15.0 | 16 | 0.0 |
| Vancomycin | >16 | 40 | 0.0 | 16 | 0.0 |

Conclusion: These results confirm that raw meat is contaminated with pathogenic bacteria, mainly *Salmonella* spp., and consequently with these raw products pathogens and resistant strains will enter the kitchen and may lead to food borne diseases and/or transfer of resistant strains or resistance genes to humans. Therefore, the prevention of food borne diseases and resistance transfer is mainly in the hands of those preparing food. These persons should as well be educated on the basic sanitary principles of food preparation as well as be informed on the possible presence of pathogens in raw products, for instance by informative labels.

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PREHARVEST INFLUENCE ON SALMONELLAE HUMAN HEALTH COSTS AND RISKS FROM PORK

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Summary: The purpose of this study is to understand the implications of the prevalence of salmonellae in pigs preharvest for the food safety risks and human health costs for humans. Mean costs from human cases of salmonellae predicted by the model was 77,183,000 \$, with a range of 6,019,000 - 723,777,000 \$. Using sensitivity analysis, on-farm apparent prevalence has an important influence on pork-associated human health costs. Tornado graphs are a poor way to assess model variable importance, and should only be used for preliminary analysis with a flow model of this type. Mismatched data sources, and

lack of data for linking prevalence to degree of contamination make risk assessment very difficult, and results are tenuous at this time. Nonetheless, we believe post-harvest handling of pork products during processing and fabrication, cooking, on-farm prevalence, and increases at transport and lairage are all important determinants of pork-associated salmonellae human health costs.

Keywords: Risk assessment, farm-to-fork, human health, pigs

Introduction: Food borne illness in the U.S., particularly illness believed to be associated with eating foods of animal origin, has received increased research and media attention. Of the zoonotic pathogens in pork, salmonellae are some of the most important. The economics of salmonellae mitigation strategies has been minimally researched. The purpose of this study is to understand the implications of the prevalence of salmonellae in pigs preharvest for the food safety risks and costs for humans.

Materials and Methods: We are developing an integrated model which uses @Risk and estimates the risk and economic impact of salmonellae in humans derived from pork. This model consists of seven modules of the farm-to-fork pork chain in the U.S. These modules estimate pig/carcass prevalence, degree of contamination 1) on-farm, 2) after transport and lairage, 3) during carcass processing to the point post-carcass chilling, 4) at fabrication and retail, and estimate human risk/costs of 5) consumer cooking and consumption, 6) salmonellae health consequences, and 7) economics of these health consequences. We use parameter estimates and data from the scientific literature throughout the model. The model works on a flow basis, with output estimates from one segment of the model generally serving as input estimates for the next segment, and assumes that all of the contamination found at the various stages originated with the pig on-farm. When possible and appropriate, we treat inputs as distributions rather than point estimates.

The mean prevalence of salmonellae in pigs at the farm uses USDA, APHIS VS CEAH CAHM (1997). Adjustments for apparent prevalence are made to derive true prevalence using sensitivity and specificity (Funk et al., 2000). We assume prevalence increases from the combined effects of transportation and lairage estimated by averaging scientific study results (from Hurd et al., 2001; McKean et al., 2001; Proescholdt et al., 1999). We assume a 1/1 correspondence between fecal positive and carcass surface positive. This is substantially more than 2/1 found by Morgan et al., 1987.

There are a large number of steps in the processing of pork carcasses that have been modeled including scalding, dehairing/singeing, carcass washing, evisceration, carcass rinse post-evisceration, steam pasteurization, and chilling. We compare results derived from the model with various published estimates (USDA, 1996, and 2003) of carcass prevalence at the point of post-chilling.

Increases in prevalence of salmonellae that occur during fabrication and at the retail level are assumed based on data from Duffy (2001). The degree of contamination is also affected by cooking by the consumer. Cooking effects and food handling care are assumed to protect against exposure differently between the two risk groups. Not all consumers eat pork (Miller and Unnevehr, 2001); 7.6 % of the population is assumed not to eat pork.

Human risks and the associated health costs are estimated which can be attributed to pork using literature that documents risks and costs from salmonellae infection. Specifically, the dose response model outlined by WHO (2002) was used. This model uses a beta-Poisson function with $a = 0.1324$, and $\beta = 51.45$, with an associated distribution around the curve, and a and β are assumed the same for low and high risk groups (WHO, 2002). Costs for human salmonellae cases were assumed to be 482.26 \$, 1032.12 \$, 11,812.19 \$, and 500,923.23 \$ for no visit to a physician, physician visit, hospitalization, and death respectively (Buzby, 1996). We assume no development of immunity; so exposure by one

person 10 times to contaminated pork results in the same number of cases as exposure by 10 people one time each to contaminated pork.

The relative importance of the various elements within the model are ranked using a tornado graph in @Risk. Sensitivity analyses are also conducted.

Results: Mean costs from human cases of salmonellae predicted by the model was 77,183,000 \$, with a range of 6,019,000 - 723,777,000 \$. Preliminary results from the tornado graph of total costs suggested that farm prevalence is one of the lesser important variables influencing costs from salmonellae in humans. Similarly, the influence of transportation and lairage was of lesser importance; preharvest influence was driven by the assumptions relative to expansion of prevalence (directly related to farm prevalence and time) spent in transportation and lairage. The impact of preharvest prevalence on human risks and costs was influenced by the degree of sanitation in slaughter and processing and the impact of cooking assumed. The errors (mistakes compared to correct ideal process and cooking) which can occur in processing (from fecal contamination) and home preparation (from cross contamination) and cooking (inadequate temperature) appeared to be more important influences on the human health costs of salmonellae than are farm prevalence, at least for the levels of prevalence seen in U.S. pig farms based on the tornado graph.

However, the tornado graph for a flow model of this type is of little use in ranking the importance of various variables. Tornado analysis suggests that the numbers of salmonellosis cases in humans in the two risk groups are the two most important variables. These variables are closest to the calculations of total costs in terms of basic model flow. Thus the tornado graph approach fails to recognize from a biological perspective that the reason these cases occur is because of inputs that occur earlier in the flow of salmonellae in the farm-to-fork chain.

This suggests that sensitivity analysis using this model is a much more appropriate approach in evaluating the influence of model variables on the human health costs. Using sensitivity analysis, on-farm apparent prevalence has an important influence on pork-associated human health costs. An increase in apparent prevalence from roughly 0.06 to 0.09 increases human health costs by 62%. Thus, the estimates for on-farm prevalence are important using this model.

Comparison of USDA estimates of prevalence post-chilling and what is suggested by the model which examines various steps in processing and fabrication resulting in log increases and decreases of surface organisms currently do not give comparable results.

Discussion: There are a number of very large problems and heroic assumptions that were made in this salmonellae risk assessment for pork. First, there are limited or no data that allows for translation between prevalence estimates and quantitative estimates of the amount of organism contamination (e.g. CFU/g, or CFU/cm²). Much of the literature collected on processing reports log reductions in surface organisms; but the vast majority of the literature prior to processing reports data on a pig or carcass prevalence basis, with the proportion positive being the reported data. This mismatch of various information sources creates substantial problems. Second, the data relating fecal positive status to surface positive status is very limited. Thus, we assume a one-to-one correspondence which potentially over estimates surface prevalence. Third, to determine human illness, an estimate of the amount of human exposure is needed. This requires translation from prevalence into a quantitative degree of exposure. Fourth, even though we believe that quantitative risk assessment models should include environmental influences, feedback loops and other elements that reflect likely circumstances (Barber et al., 2003), we assume a simple straight flow model with all risk derived originally from pigs. Finally, almost all data are limited and extremely important assumptions that influence model output to varying degrees must be made at every stage of the analysis.

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There is disagreement between large and knowledgeable groups of scientists about very basic points important to a salmonellae risk assessment in pork. USDA (1998) argues strongly for a difference between high and low risk groups of people in terms of susceptibility to salmonellae infection, and they propose different betas. WHO (2002) argue that "...it is not possible to conclude that some segments of the population are more susceptible to ... *Salmonella*...". However, WHO (2002) does suggest that severity of illness can be different for different risks groups.

There may indeed be differences by serotype in dose-response, severity of illness, etc., for humans (Schlosser et al., 2000). Different serotypes of salmonellae predominate in pigs and humans. This model considers all salmonellae as a group and makes no distinctions based on serotype. Additionally, there are important differences in microbiological identification of salmonellae in each of the studies referenced; such differences also have important implications (Maddox, 2003) for the model. These implications are unknown and not explicitly considered.

There is a large body of literature needed to support this risk assessment. We have 323 references in our database that supports details in the model. Only a small fraction of some of the more important studies and references is listed in this paper.

Despite model deficiencies and lack of data, food safety risk assessment models still yield some insights into process control, evaluation, and data collection priorities (Roberts et al., 1999). We hope this is also the case with this model.

In conclusion, post-harvest handling of pork products during processing and fabrication, cooking, on-farm prevalence, and increases at transport and lairage are all important determinants of pork-associated salmonellae human health costs.

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PERFORMANCE OF ANTI-SALMONELLA LACTIC ACID BACTERIA IN THE PORCINE INTESTINE

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Summary: Of five anti-*Salmonella* porcine cultures administered to pigs at 10¹⁰ cfu/day, two *Lactobacillus murinus* strains demonstrated superior survival during gastrointestinal transit. Both were detected at 10⁷-10⁸ cfu/g faeces which was higher (P<0.05) than *Pediococcus pentosaceus* DPC6006 (10⁶ cfu/g). One *Lb. murinus* strain was also excreted at higher numbers (P<0.05) than either *Lb. salivarius* DPC6005 or *Lb. pentosus* DPC6004 (both 10⁶ cfu/g). The *Lb. murinus* strains persisted in both the faeces and the caecum for at least 9 days post-administration. Animals fed a combination of all five strains at 10¹⁰ cfu/day excreted 10⁷ cfu/g of the administered strains, which was higher (P<0.05) than only *P. pentosaceus* DPC6006. Randomly amplified polymorphic DNA (RAPD) PCR analysis revealed that both *Lb. murinus* strains predominated in the faeces of these animals during administration, while post-administration, both *Lb. murinus* strains and *Lb. pentosus* DPC6004 were recovered from the faeces and the caecum while *P. pentosaceus* DPC6006 was only detected in the caecum. After 21 days of culture administration, faecal *Enterobacteriaceae* counts were reduced in pigs fed *Lb. salivarius* DPC6005, *P. pentosaceus* DPC6006, *Lb. pentosus* DPC6004 and the culture mix, though not significantly. Overall, the porcine intestinal isolates offer potential as probiotics for enteropathogen reduction in pigs; possibly as a combination due to strain variation.

Keywords: probiotic, pigs, gastrointestinal tract, *Lactobacillus*, *Pediococcus*

Introduction: Probiotics aimed at restoration and maintenance of a healthy gut microflora offer potential as a means of controlling enteric pathogen carriage in pigs. Competitive exclusion cultures are particularly effective in pigs (Nisbet, 2002). However, uncertainty regarding their exact composition has led to concerns that they may result in pathogen transmission and could also hinder regulatory approval. Alternatively, defined probiotic strains, most commonly lactic acid bacteria, *Bacillus* or yeast, can be used as feed additives, either individually or as mixtures. However, commercial animal probiotic products do not always contain the strains or species listed on the label at an adequate dose or may lack evidence of a probiotic effect (Weese, 2002), highlighting the need for proper

selection and characterisation of strains intended for probiotic use. Furthermore, although interest is increasing in the use of probiotics as microbial feed additives for pathogen reduction in pigs, relatively few strains have demonstrated efficacy *in vivo*. The objective of this study was to investigate intestinal performance of five potentially probiotic porcine caecal isolates with anti-*Salmonella* activity (Casey *et al.*, 2003), when orally administered both individually and as a five-strain combination.

Materials and Methods: Twenty eight pigs weaned at 24-28 days were blocked on sex and weight and assigned at random to one of seven treatments (n=4): (1) control (no culture), (2) *Lb. salivarius* DPC6005, (3) *P. pentosaceus* DPC6006, (4) *Lb. pentosus* DPC6004, (5) *Lb. murinus* DPC6002, (6) *Lb. murinus* DPC6003 and (7) mixture of all five cultures. Rifampicin resistant (Rif^r) variants of each strain, required for enumeration were administered as skim milk fermentates containing 10^8 cfu/ml. The feeding trial consisted of three consecutive periods; 10 days baseline (100 ml sterile skim milk supplemented with 0.5 % (w/v) yeast extract per day), 21 days culture administration (100 ml fermentate providing $3 \sim 10^{10}$ cfu/day relevant strain(s) for treatments 2-7 and 100 ml lactic acid-acidified sterile skim milk per day for control pigs) and 9 days post-administration (no milk or culture). Animals also had unrestricted access to water and non-medicated creep feed. Faecal samples were obtained during the baseline (day -5), administration (days 3, 8, 15 and 22) and post-administration (day 26) periods. At day 30 two pigs per treatment were sacrificed and the caecal contents sampled. Faecal and caecal samples were homogenised in maximum recovery diluent as 1:10 dilutions, further diluted and pour-plated. MRS agar containing 100 mg/ml of rifampicin (MRS-RIF) incubated anaerobically at 37 °C for 2 days was used to enumerate the administered strains, while *Enterobacteriaceae* were enumerated on violet red bile glucose agar incubated at 37 °C for 24 h. Representative colonies from MRS-RIF plates from faecal and caecal samples were genetically fingerprinted by RAPD PCR as described previously (Gardiner *et al.*, 1998). The data were statistically analysed using Genstat and Tukey's Test was used for separation of means.

Results: During the 21-day administration period all animals excreted 10^5 - 10^8 cfu/g of the administered strains (Table 1), as confirmed by genetic fingerprinting of representative faecal isolates using RAPD PCR. However, the *Lb. murinus* strains DPC6003 and DPC6002 demonstrated superior survival, yielding the highest mean faecal excretion of all individually administered strains (Table 1) and accounting for 21-24 % of total faecal lactobacilli. The mean counts for both *Lb. murinus* strains during the 21-day administration period (day 3-22) were higher (P<0.05) than those of *P. pentosaceus* DPC6006, which had the lowest mean faecal count (Table 1). The mean faecal day 3-22 count of *Lb. murinus* DPC6003 was also higher (P<0.05) than *Lb. salivarius* DPC6005 and *Lb. pentosus* DPC6004 (Table 1), both of which represented on average only 1.1-1.3 % of total faecal lactobacilli. Animals fed the five-strain combination excreted high counts of the administered strains and the mean count during the administration period was higher (P<0.05) than that of *P. pentosaceus* DPC6006 (Table 1), with both *Lb. murinus* strains or *Lb. murinus* DPC6003 alone predominating, as determined by RAPD PCR (data not shown). Although faecal counts of the administered strains declined once administration stopped, all animals fed the *Lb. murinus* cultures or the strain combination still harboured high levels of the administered strains at day 5 post-administration (Table 1), with the predominating isolates in the mixture-fed animals identified by RAPD as either *Lb. murinus* DPC6002 or DPC6003 or *Lb. pentosus* DPC6004 (data not shown). These treatment groups also harboured highest levels of the administered strains (10^3 - 10^6 cfu/g) in the caecum 9 days post-administration, with the *Lb. murinus* strains predominating in one of the mixture-fed animals sacrificed and *Lb. pentosus* DPC6004 and *P. pentosaceus* DPC6006 in the other (data not shown). In comparison, strains DPC6006, DPC6004 and DPC6005, when administered individually persisted less well in both the faeces and the caecum.

Table 1. Mean faecal counts (log cfu/g) of administered strains in pigs fed $\sim 10^{10}$ cfu/day of skim milk fermentates of each of the porcine cultures singly or as a combination

| Treatment ^a | Day 3 | Day 8 | Day 15 | Day 22 | Day 26 (Day 5 post- administration) | Day 3-22 (Administration period) |
|-------------------------------|-------|--------------------|--------------------|--------------------|---|--|
| <i>Lb. salivarius</i> DPC6005 | 7.08 | 6.93 ^c | 6.60 ^{ab} | 6.37 ^{ab} | 4.15 ¹ | 6.75 ^{bc} |
| <i>P. pentosaceus</i> DPC6006 | 6.56 | 5.52 ^d | 5.39 ^b | 5.61 ^b | 4.60 ² | 5.77 ^c |
| <i>Lb. pentosus</i> DPC6004 | 6.81 | 7.31 ^{bc} | 6.53 ^{ab} | 6.21 ^{ab} | 5.55 ³ | 6.72 ^{bc} |
| <i>Lb. murinus</i> DPC6002 | 7.04 | 8.30 ^{ab} | 7.72 ^a | 7.66 ^{ab} | 5.39 | 7.68 ^{ab} |
| <i>Lb. murinus</i> DPC6003 | 7.74 | 8.38 ^a | 8.29 ^a | 8.10 ^a | 6.45 | 8.1 ^a |
| Culture combination | 7.57 | 8.01 ^b | 8.22 ^a | 7.85 ^{ab} | 7.29 ² | 7.91 ^{ab} |

^{a,b,c,d} Means within the same column showing different superscripts are significantly different ($P < 0.05$)

^{1, 2, 3} Administered strains detected in only two pigs, three and one pig, respectively

On average, when all treatment groups were analysed together faecal *Enterobacteriaceae* were lower ($P < 0.05$) towards the end of the culture administration period (day 15, 22 and 26) than prior to or during the first week (day -5, 3 and 8) (data not shown). However, no significant effects on faecal *Enterobacteriaceae* were obtained when the entire culture administration period (day 3-22) was analysed, probably because counts were highly variable within individual treatment groups and fluctuated throughout the trial. However, at day 15 mean faecal *Enterobacteriaceae* were lower ($P < 0.05$) in animals fed *Lb. murinus* DPC6003 than in animals fed *Lb. murinus* DPC6002 or the culture mix (Table 2). Furthermore, when mean pre-administration counts were compared with counts after 21 days of culture administration, 87-98 % reductions in *Enterobacteriaceae* were obtained in pigs fed *Lb. salivarius* DPC6005, *P. pentosaceus* DPC6006, *Lb. pentosus* DPC6004 and the culture mix (Table 2), although the results were not statistically significant. Counts also decreased by 83 % in the control group fed acidified skim milk.

Table 2. Effect of culture administration on mean faecal *Enterobacteriaceae* counts (log cfu/g) in pigs

| Treatment | Day -5 count (Pre- administration) | Day 15 count | Day 22 count (After 21 days culture administration) | % Reduction after 21 days culture administration ¹ |
|-------------------------------|--|--------------------|---|---|
| Control | 7.39 | 6.21 ^{ab} | 6.63 | 83 |
| <i>Lb. salivarius</i> DPC6005 | 7.42 | 5.71 ^{ab} | 5.67 | 98 |
| <i>P. pentosaceus</i> DPC6006 | 6.87 | 6.06 ^{ab} | 6.0 | 87 |
| <i>Lb. pentosus</i> DPC6004 | 8.10 | 6.26 ^{ab} | 6.58 | 97 |
| <i>Lb. murinus</i> DPC6002 | 6.24 | 7.31 ^a | 6.59 | 0 ² |
| <i>Lb. murinus</i> DPC6003 | 6.56 | 5.21 ^b | 6.69 | 0 ² |
| Culture mix | 7.22 | 6.94 ^a | 5.74 | 97 |

^{a,b} Means within the same column showing different superscripts are significantly different ($P < 0.05$)

¹ $(N_0 - N/N_0) \times 100$, where N_0 = mean day -5 count and N = mean day 22 count (both cfu/g faeces)

² Counts increased in these treatment groups

Discussion: Survival during intestinal transit and colonization of the gut are important criteria for orally administered probiotics. The strains isolated in our laboratory performed well, especially *Lb. murinus*, which accounted for a large percentage of total faecal lactobacilli, in comparison with previously administered lactobacilli (24 % vs. 2.5 %) (Pedersen *et al.*, 1992). The strains administered persisted, albeit to varying degrees, in the faeces and the caecum for at least 5 and 9 days post-administration, respectively, which compares well with previous reports of 3-7 days faecal persistence for other lactobacilli (Pedersen *et al.*, 1992). These findings demonstrate that the porcine isolates compete effectively with the native microflora and become established in the intestine to some extent. The strain variation with respect to survival and persistence in the porcine gut has previously been observed (Pedersen *et al.*, 1992), suggesting that there may be advantages to feeding a culture mix. Indeed, individual strains within

a mix survived differently in individual animals and in general, the culture combination resulted in good survival and persistence, as previously observed by Pedersen *et al.* (1992). Many studies have reported reductions in intestinal coliform and *Enterobacteriaceae* due to probiotic administration; however, some have seen no effects (Simon *et al.*, 2003). In the present study most of the cultures resulted in reductions in faecal *Enterobacteriaceae*; in fact, reductions of up to 98 % were observed. However, except for day 15, these reductions were not statistically significant, probably due to the variation in counts between individual animals, a common observation in probiotic animal trials. This inconsistency may be explained by individual variations in response to probiotics due to the complexity of the intestine (Simon *et al.*, 2003). Future experiments using larger treatment groups and deliberate *Salmonella* infection should provide further information on the pathogen-lowering ability of these cultures.

Conclusions: Pig-derived potentially probiotic cultures with anti-*Salmonella* activity can be effectively delivered to the porcine intestine by oral administration, either individually or as a strain combination. However, it was evident that certain cultures survived at higher levels, persisted for longer in the caecum post-administration and were more effective in reducing pathogenic indicator species, highlighting the advantages of using combination probiotics in pigs. We conclude that, although further characterisation of efficacy is necessary, the findings provide a basis to further explore the potential of these porcine isolates as microbial feed additives (most likely administered as a mixture) for *Salmonella* reduction in pigs.

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Survival of *Salmonella* serovar Typhimurium inside porcine monocytes is associated with complement binding and suppression of the production of reactive oxygen species

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Summary: Macrophages are thought to play a major role in the development of *Salmonella* carriers in swine. It was the aim of the present study to characterize the interactions of a *Salmonella* serovar

Typhimurium strain with porcine peripheral blood monocytes. The production of reactive oxygen species (ROS) by monocytes and the numbers of intracellularly killed bacteria differed significantly between the different pigs used. Opsonization of *Salmonella* bacteria with complement significantly decreased bacterial killing. Interestingly, monocytic ROS production was suppressed by metabolically active bacteria. In conclusion, binding to host complement and suppression of monocyte ROS production enable ser. Typhimurium to survive for at least 6 hours in porcine monocytes. Moreover, individual differences of porcine monocytes to produce ROS and to kill the intracellular *Salmonella* bacteria might account for the development of the carrier state in some pigs and not in others.

Keywords: *Salmonella* Typhimurium, pig monocyte, carrier, reactive oxygen species

Introduction: Persistent *Salmonella* infections in pigs result in contamination of carcasses in the slaughterhouse. The mechanism of this carrier state is poorly understood but is associated with survival of *Salmonella* bacteria inside host macrophages. However, studies on interactions of porcine mononuclear cells with *Salmonella* are scarce (Riber and Lind, 1999). It was therefore the aim of the present study to characterize the following interactions of *Salmonella* serovar Typhimurium with porcine monocytes: 1) production of reactive oxygen species 2) production of reactive nitrogen intermediates 3) the formation of spacious phagosomes (unusually wide, *Salmonella* containing endosomes) 4) bacterial killing and 5) cytotoxicity of the *Salmonella* bacteria on the porcine monocytes.

Materials and methods: Monocytes. Peripheral blood monocytes were collected from 14 to 24 week old pigs using density centrifugation on a ficoll-paque density gradient and subsequent adhesion to tissue culture flasks. Cell purity was determined using incubation with monoclonal mouse anti-SWC3 antibodies and flow cytometry. A purity of 85-90 % was obtained.

Salmonella strain. A serovar Typhimurium strain (20735c) isolated from pigs was used throughout the studies. Bacteria were grown for 6 h at 37 °C in Brain Heart Infusion, washed three times and resuspended in Hank's balanced salt solution (HBSS) at the desired concentration. Viable bacteria were opsonized with guinea pig complement (Virion Ltd., Switzerland) during 30 min at 20 °C. Bacteria were inactivated using either UV light or acetone. In order to abolish protein synthesis, *Salmonella* bacteria were incubated with 25 µg/ml chloramphenicol.

Production of reactive oxygen species by porcine monocytes after stimulation with *Salmonella* Typhimurium. Production of reactive oxygen species (ROS) was determined using luminol-enhanced chemiluminescence (CL). Briefly, 10⁶ monocytes were seeded per well containing 200 µM luminol and exposed either to viable, inactivated or chloramphenicol treated bacteria at 10 bacteria per monocyte or to phorbol myristate acetate (PMA) at 20 µg/ml. Viable bacteria were used either native or opsonized with guinea pig complement. CL was recorded during 1 h. At 1 h after stimulation with *Salmonella*, the residual monocyte activity was assessed by the addition of 20 µg/ml PMA and recording of CL for another hour. The test was performed on monocytes from 4 pigs and repeated three times in triplicate.

Production of reactive nitrogen intermediates by porcine monocytes after stimulation with *Salmonella* Typhimurium. Production of reactive nitrogen intermediates (RNI) was determined using the Griess reaction. Briefly, 10⁶ monocytes were seeded per well and inoculated with 10⁷ *Salmonella* bacteria. After centrifugation at 364 x g for 10 min at 37 °C and subsequent incubation for 30 min at 37 °C, the culture medium was replaced by medium containing 10 µg/ml gentamicin to kill extracellular bacteria. After 24 h of incubation at 37 °C, 100 µl of supernatant was collected per well and the Griess reaction was performed. The test was performed on monocytes from 4 pigs and repeated three times in triplicate.

Spacious phagosome formation in porcine monocytes after stimulation with *Salmonella* Typhimurium. The formation of spacious phagosomes (SP) was determined using a previously described technique (Alpuche Aranda et al., 1995). This technique is based on the fluorescent visualization of SP by inclusion of FITC-labelled dextran and subsequent microscopic evaluation. The test was performed on monocytes from 2 pigs.

Killing of *Salmonella* Typhimurium by porcine monocytes. Monocytes were seeded at 10^5 cells per well and exposed to 10^6 *Salmonella* bacteria as described in the RNI assay. The bacteria were used either native or opsonized with guinea pig complement. At 0, 2 and 6 hours after exposure, the cells were rinsed, lysed and the number of *Salmonella* bacteria was counted by plating tenfold serial dilutions on brilliant green agar. The test was performed on monocytes from 4 pigs and repeated at least three times in triplicate.

Cytotoxicity of *Salmonella* Typhimurium on porcine monocytes. Monocytes were inoculated with *Salmonella* as described in the CL assay. After 2 h of incubation, the supernatant was collected and the level of lactate dehydrogenase activity was measured (Roche Diagnostics GmbH). The test was performed on monocytes from 4 pigs and repeated three times in triplicate.

Results:

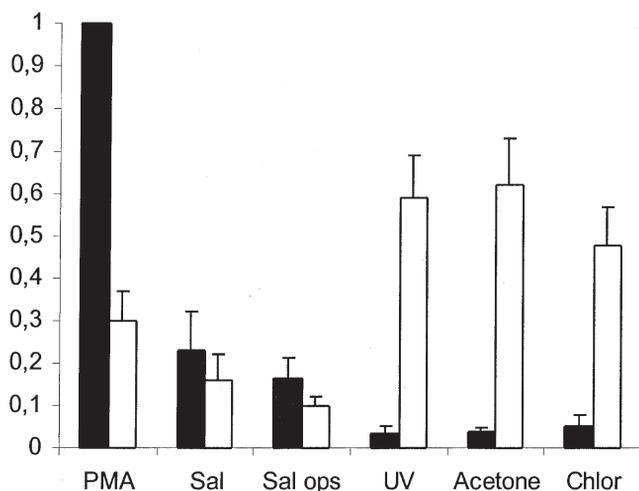


Fig. 1. Production of reactive oxygen species by porcine monocytes. The black bars represent the average chemiluminescent responses \pm s.e. of monocytes exposed to PMA, viable salmonellae (Sal), opsonized bacteria (Sal ops), UV or acetone inactivated bacteria or chloramphenicol treated salmonellae (chlor). Values are expressed as fractions of the peak value obtained after stimulation with PMA. White bars represent the average residual activity \pm s.e.

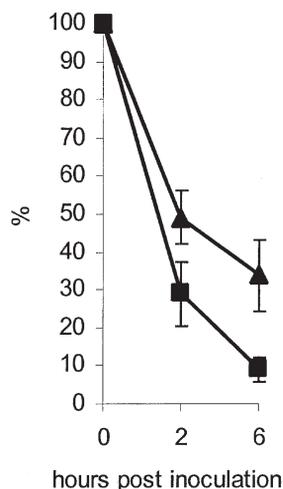


Fig. 2. Average percentage \pm s.e. of intracellular survival of *Salmonella* bacteria in porcine monocytes. Bacteria were either native (squares) or opsonized (triangles).

Production of ROS and RNI and the formation of SP by porcine monocytes exposed to *Salmonella* Typhimurium. The results of the CL assays are shown in Figure 1. Viable salmonellae induced a higher CL response compared to inactivated or chloramphenicol treated bacteria. Contrary to exposure to inactivated and chloramphenicol treated bacteria, monocytes exposed to viable bacteria showed only marginal residual activity. The monocytes from one pig produced approximately three times more ROS than those from the other three (one way ANOVA, $P < 0.05$). The *Salmonella* strain did not cause cytotoxicity in the monocytes at 2 h post inoculation. No detectable amounts of RNI were produced by the porcine monocytes in none of the test conditions. In 9-12 % of the *Salmonella* containing monocytes, spacious phagosomes were detected.

Killing of *Salmonella* Typhimurium by porcine monocytes. Results of the microbicidal assays are summarized in Figure 2. Numbers of intracellular salmonellae decreased significantly less (paired t-test, $P < 0.05$) when the bacteria were opsonized with guinea pig complement. Monocytes from one pig were significantly less capable of killing *Salmonella* bacteria between 0 and 2 h post inoculation compared to those from the other three pigs (approximately 2.5 times less; one way ANOVA, $P < 0.05$).

Discussion: Most of the pathogenesis of salmonellosis has been described in mice, chickens and calves. Few data exist on the interactions of *Salmonella* with porcine phagocytes. The lack of RNI production by the porcine monocytes demonstrates that not all data collected from mice can be extrapolated to other species. In mice, the production of NO by inducible NO synthase (iNOS) is important in controlling intracellular multiplication of *Salmonella* bacteria (Umezawa et al., 1997). In contrast with Ribber and Lind (1999), the number of intracellular bacteria steadily decreased over the 6 h period, suggesting lack of intracellular bacterial multiplication. Interestingly, opsonization with complement increased the number of surviving salmonellae. Possibly, intracellular trafficking and thus survival of the *Salmonella* bacteria might be influenced by entry in the host cell through complement receptor binding (Ishibashi and Arai, 1996). The production of ROS by host macrophages is an important first defence mechanism (Vazquez Torres et al., 2000), which *Salmonella* must circumvent in order to survive intracellularly inside the host cell. The *Salmonella* Typhimurium strain was able to suppress the production of ROS in porcine monocytes. This suppression was abolished when chloramphenicol treated bacteria were used, indicating that suppression of monocytic ROS production requires active bacterial protein synthesis. Individual differences between pigs were noticed both in the production of ROS and in the ability to kill *Salmonella*. These individual differences might account for a different course of infection, for example the development of the carrier state in some but not in other pigs.

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O 42 The stomach acts as a barrier against *Salmonella* in pigs fed a meal diet

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Summary: Finishing pigs fed a coarsely ground meal (CGM) diet showed increased *in vitro* death rate of *Salmonella* in the gastric content and a reduced number of enterobacteria in the small intestine and caecum compared with a finely ground and pelleted diet (FGP). The CGM diet resulted moreover in a slower gastric emptying rate, increased the DM content and established a pH-gradient in the stomach. This affected the microbiota in the gastric digesta resulting in more lactic acid

bacteria and fewer enterobacteria. Consequently *Salmonella* bacteria are killed in the stomach and do not enter and proliferate in other parts of the gastrointestinal tract. Furthermore the time after feeding a meal is of importance to whether or not *Salmonella* bacteria will survive transit through the stomach.

Introduction: Previous studies have repeatedly shown that feeding coarsely ground meal feed significantly reduced the incidence of *Salmonella* in finishers compared with finely ground heat-treated and pelleted feed. The occurrence of enterobacteria, such as *Salmonella*, in the gastrointestinal tract is strongly influenced by feed composition and processing. However, the mechanisms involved are not fully understood, but it has been suggested that the stomach plays a central role in preventing the establishment of *Salmonella* infections in finishing pigs (van Winsen *et al.* 2001). In the present study, the effects of feed processing on survival of *Salmonella* in gastric digesta and on the microbiota in the gastrointestinal tract with special emphasize on the stomach were investigated.

Keywords: Feed, Microbial flora, Rheological properties

Materials and Methods: Two groups of 30 commercial hybrid castrates were fed either a finely ground pelleted diet (FGP) or a coarsely ground meal (CGM). At approximately 100 kg liveweight, 6 pigs from each of the two diets were killed at 0.5, 2, 5, 8 and 9.5 h post feeding. The gastrointestinal tracts were immediately removed and divided into 5 segments: Distal and proximal stomach, small intestine, caecum and colon. The DM content, pH, SCFA and lactic acid concentration were measured in all segments, microbial enumeration was performed on content from the stomach, small intestine and caecum, and physico-chemical characterization was made of the stomach content. In addition, the gastric emptying rate was determined and the growth-/death rate of *Salmonella* DT12 in the fresh content from the distal and proximal part of the stomach was determined by an *in vitro* method.

Results and discussion: The *in vitro* method revealed a significantly higher death rate of *Salmonella* in the stomach content from pigs fed the CGM diet, Figure 1. Furthermore time post feeding affected the death rate of *Salmonella*.

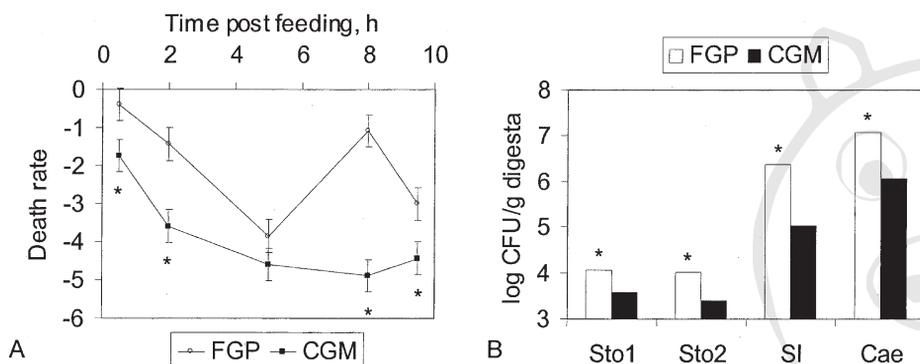


Figure 1. (A) *In vitro* death rates of *Salmonella* DT12 in gastric content and (B) density of enterobacteria in digesta from the proximal stomach (Sto1), distal stomach (Sto2), small intestine (SI) and the caecum (Cae). For both graphs: at different time post feeding in pigs fed a finely ground and pelleted (FGP) or a coarsely ground meal (CGM) diet. * indicates significant difference, $P < 0.05$.

In the pigs fed the CGM diet, a higher DM content was found in the gastric digesta, Figure 2. This might be one of the explanations for the clear pH-gradient that was determined in stomachs of pigs fed the CGM diet. Also it is noteworthy that the pH in the gastric digesta of the pig offered the FGP diet was significant higher compared with the pH in the distal stomach of the pigs fed the CGM diet for

more than 2 h post feeding. Moreover diet and time post feeding affected the bacterial populations in the stomach, Figure 2. In pigs receiving the CGM diet, more lactic acid bacteria were found in the proximal part of the stomach. In pigs fed the FGP diet, a higher density of enterobacteria was observed until 5 h post feeding in the gastric digesta. It is furthermore likely that the slower gastric emptying rate found in the CGM fed pigs also influenced the microbial eco-system in the stomach.

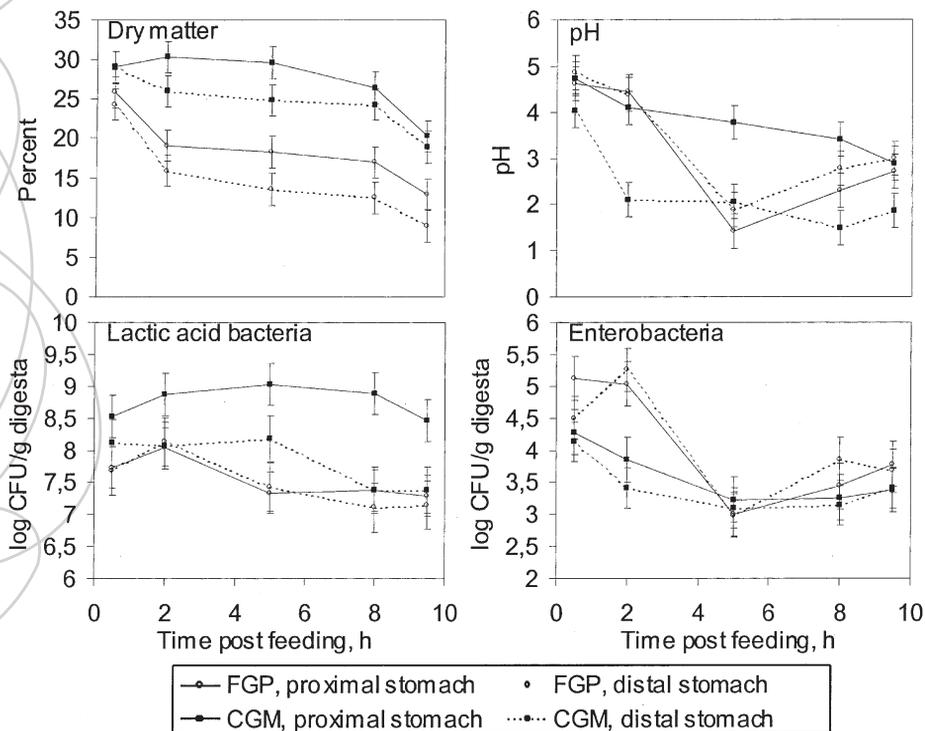


Figure 2. Dry matter, pH, lactic acid bacteria and enterobacteria in gastric digesta from the proximal and distal part of the stomach at different time post feeding in pigs fed fed a finely ground and pelleted (FGP) or a coarsely ground meal (CGM) diet.

In the small intestine and caecum of the pigs given the CGM diet, the number of enterobacteria was reduced compared with the pigs fed the FGP diet, Figure 1. This indicates that feeding CGM diets to pigs prevents *Salmonella* bacteria from proliferating in the lower parts of the gastrointestinal tract. The stomach acts as a barrier preventing harmful bacteria from entering the lower part of the gastrointestinal tract. Furthermore this study indicate, that in pigs fed a FGP diet, the time after a meal where *Salmonella* bacteria are able to survive passage through the stomach is prolonged compared with the pigs fed a CGM diet.

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Zoonotic pathogens and antimicrobial resistance in 'animal-friendly' pig production systems in Switzerland

O 43

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Keywords: Salmonella, Campylobacter, Yersinia, welfare, food safety

Summary: In a cross-sectional study, the impact of 'animal-friendly' housing systems on the prevalence of *Salmonella* species, *Campylobacter* species, and *Yersinia enterocolitica* in finishing pigs and pork was investigated. Furthermore, antimicrobial resistance patterns of isolated campylobacter strains were analysed. In faecal samples of two out of 88 fattening pig farms salmonellae were isolated. All 865 samples of pork were found to be negative. Campylobacter was isolated on 98.9 % of the farms but only from 0.2 % of the pork samples. Yersinia were found in samples of 63.3 % of the farms and in 15.4 % of pork samples. For all three bacteria, there was no statistically significant difference in the prevalence between conventional and 'animal-friendly' housing systems. In 'animal-friendly' farms, antimicrobial resistance of campylobacter isolates to fluoroquinolones and streptomycin was significantly less frequent than in conventional farms. Furthermore, fewer isolates had resistance to three or more antimicrobials in 'animal-friendly' farms.

Introduction: 'Animal-friendly' production systems for finishing pigs are promoted by the Swiss Federal Office for Agriculture. Such systems primarily differ from conventional production in the use of straw bedding and outdoor access. In 2000, 28 % of Swiss fattening pigs were kept in farms with 'animal-friendly' production systems (Anonym, 2002). It was shown that these systems improve pig welfare (Cagienard et al., 2002), but they might represent an increased risk for the occurrence of zoonotic pathogens in pigs and pork. Furthermore, there are few data on the effect of these housing systems on antimicrobial resistance of zoonotic pathogens, specifically *Campylobacter* species.

The objective of the study was to investigate the prevalence of *Salmonella* species, *Campylobacter* species and *Yersinia enterocolitica* in finishing pigs and pork in Switzerland. In addition, the effect of 'animal-friendly' housing systems on the load of these pathogens in pigs and pork and on antimicrobial resistance in isolated campylobacter strains was evaluated.

Materials and methods: A cross-sectional study was performed on 88 finishing pig farms with either conventional (41 farms) or 'animal-friendly' (47 farms) housing systems. On each farm, 20 faecal samples were taken. Five samples were combined to make one pooled sample (Steger et al., 2000) and cultured for *Campylobacter* species, *Salmonella* species, and *Yersinia enterocolitica*. At retail, 865 samples of fresh pork, produced either conventionally (48 % of the samples) or 'animal-friendly', were collected in all regions of Switzerland. Culture was performed for all three micro-organisms using standard techniques. For antimicrobial resistance testing, one strain from each campylobacter-positive sample was selected and the disc diffusion method was used (NCCLS, 1997, 1998). Tested antimicrobials included fluoroquinolones, erythromycin, tetracycline, kanamycin, polymyxin, chloramphenicol, gentamicin and streptomycin.

Results: *Salmonella* species were found in samples from two farms (2.3 %). However, all 865 samples of pork at retail were found to be negative. In samples from all but one finishing farms, *Campylobacter* species were isolated. On retail level, only 0.2 % of the pork samples were found positive for *Campylobacter* species. *Yersinia enterocolitica* were isolated in samples from 63 % of the farms, but only in 15 % of pork samples. In pork, 119 out of 133 (89.5 %) yersinia isolates belonged to biotype 1A. Six isolates (4.5 %), belonging to several bio- and serotypes, were considered to be potential human pathogens, and eight

isolates (6 %) could not be further classified. For all three cultured bacteria, there was no statistically significant difference in the prevalence between conventional and 'animal-friendly' housing systems. Statistically significant differences between the two housing systems were observed for resistance to fluoroquinolones and streptomycin. Resistance to fluoroquinolones was present in 31 % of the isolates from traditional farms, but only in 20 % of the isolates from 'animal-friendly' farms. Resistance to streptomycin was common in both housing systems, but more frequent in traditional farms (85 % of the isolates) than in 'animal-friendly' farms (74 % of the isolates). When resistance to multiple antimicrobials was compared, fewer isolates from 'animal-friendly' farms were resistant to three or more of the tested antimicrobials.

Discussion: Our results document that for prevalence of salmonella, campylobacter and yersinia, microbiological quality of Swiss pork was on an equally high level in both conventional and 'animal-friendly' production systems. Thus, the endeavour of Swiss government and Swiss pork industry to combine 'animal-friendly' meat production with food safety was shown to have been successful. As regards antimicrobial resistance, the quality of 'animal-friendly' produced meat was even superior to conventional production. It was shown in another study on the same farms, that pigs in 'animal-friendly' farms had less injuries of the joints and injuries from tail biting than pigs in traditional farms (Cagienard et al., 2002). Fewer injuries might have resulted in a reduced use of antimicrobials. Therefore, reduced antimicrobial resistance in 'animal-friendly' production systems was probably not directly caused by differences in the housing systems, but it could be interpreted as an effect of improved pig health and therefore fewer treatments. This hypothesis will have to be investigated further.

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O 44 *Campylobacter* species distribution in outdoor pigs

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Keywords: hippuricase gene, DIG nucleotide probe, colony hybridization,

Summary: A colony blot hybridization method with a digoxigenin-(DIG)labelled nucleotide probe was developed to enable *Campylobacter jejuni* species-specific identification of colonies present

in minority. *C. jejuni* is the dominant cause of human cases of Campylobacteriosis. In contrast, *C. coli* is normally dominant in conventional pigs but it can be speculated that outdoor pigs host more *C. jejuni* due to a closer contact to the environment and wild-life where *C. jejuni* is pre-dominant. Since *C. jejuni* is considered more virulent to humans a potential shift towards *C. jejuni* in pigs may infer an importance to food safety. Individual pigs are often colonized with several *Campylobacter* strains. However, with conventional culturing techniques only few colonies are typically identified to the species level and thus *C. jejuni* will only rarely be isolated if present in minority. Using the hybridization method it was possible to identify *C. jejuni* colonies in 4 out of 20 faecal samples from finisher pigs.

Materials and Methods: *DIG-labelled C. jejuni specific nucleotide probe:*

A primer-pair termed HIP400F and HIP1134R for amplification of *C. jejuni* (a 735 bp amplicon) was previously designed on basis of the sequence of the hippuricase (*hip*) gene, which is absent from campylobacters other than *C. jejuni*. These primers were used (1 μ M each) to prepare a *C. jejuni* specific nucleotide probe labelled with digoxigenin (DIG) using the PCR DIG Probe synthesis kit (Roche). For preparation of PCR mastermix the PCR DIG probe synthesis mix containing 0.7 mM DIG-11-dUTP was diluted 1:3 with the unlabelled dNTP stock solution. Two microlitre of a crude DNA boil-lytate of the *Campylobacter jejuni* CCUG 11284 strain was used as template DNA in the PCR. The PCR thermocycler conditions were 94^A for 5 min; 30 cycles of 1 min at 94^AC, 1 min at 62^AC and 2 min at 72^AC and final extension for 8 min at 72^AC.

Colony blot hybridisation: A colony lift was made by placing a nylon membrane on an agar plate with single campylobacter colonies (<150 cfu) for 1 min. The membranes were further processed and hybridized with the DIG-labelled nucleotide probe according to colony hybridization protocol from Roche with chromogenic detection of the target-probe hybrids. The required reagents were also purchased from Roche. The pre-hybridization (1 h) and hybridization (overnight) steps were carried out at 60^AC.

Validation of the method: Rectal faecal samples from 10 finisher pigs (conventional) were screened for the presence of *C. coli* and *C. jejuni* with a species-specific real-time PCR (rt-PCR) (in-house test). A ten-fold dilution series was prepared by dilution of 1 g of faecal material in 9 ml of Bolton selective enrichment broth (without blood) and 0.1 ml of each dilution was spread on modified charcoal-cefoperazone-deoxycholate agar (mCCDA) plates before incubation. All incubations were performed in a microaerobic atmosphere at 41.5^OC for two days. Then 2 μ l of a crude DNA boil-lytate prepared from 1 ml of the pre-enriched Bolton broth was tested in the rt-PCR to screen for the presence of *Campylobacter jejuni* and *C. coli*, respectively. Bacterial colonies are required for hybridization and isolation of strains but the colony lift can be performed either from the direct spread on mCCDA (before pre-enrichment) or from new mCCDA plates made from the pre-enriched broth of *C. jejuni* positive sample-dilutions. However, it is essential to obtain plates with single colonies of *Campylobacter*. In this case, 0.1 ml of the enrichment broth from the *C. jejuni* positive (rt-PCR) sample were spread on mCCDA plates. After incubation, plates with less than 150 single colonies were subjected to colony hybridization as described above. Another 10 faecal samples from finisher pigs were diluted 10-fold and spread directly on mCCDA plates for colony hybridization without rt-PCR screening for *C. jejuni* beforehand. When a target-probe hybrid was identified, presumptive *C. jejuni* colony material from the re-incubated mCCDA plate was subcultivated. The resulting isolates were biochemically tested for hydrolysis of hippurate, which distinguishes *C. jejuni* from other *Campylobacter* species. Furthermore, the isolates were identified with rt-PCR.

Results: The rt-PCR showed the presence of *C. coli* in all and *C. jejuni* in 2 of the 10 pre-enriched faecal samples, although one sample only yielded a weak *C. jejuni* amplification signal. The following colony hybridization of the positive sample showed that approximately half of the more than 100 cfu was target-probe hybrids. Six of the nine isolates obtained from the presumptive *C. jejuni* colonies on this mCCDA plate were tested hippurate-positive and verified as *jejuni* in the rt-PCR test. Whereas three of the *jejuni* presumptive isolates were hippurate-negative and identified as *C. coli* (rt-PCR). No target-probe hybrids were obtained from the sample with a weak *C. jejuni* amplification signal in the rt-PCR or from the negative samples.

Three of the ten samples used for direct colony hybridization showed target-probe hybrids on the colony blot membrane. These samples showed 3, 5 and 1 hybrids out of 127, 58 and 145 cfu, respectively. The isolates obtained from the identified colonies were confirmed as *C. jejuni* in rt-PCR and all but one isolate were tested hippurate-positive.

Discussion and conclusions: In the current study, a species-specific DIG-labelled nucleotide probe has been developed and it was possible to identify few *C. jejuni* among a majority of other *Campylobacter* spp. on mCCDA plates. However, three isolates from target-probe hybrids could not be confirmed as *C. jejuni* (*C. coli*) but this could probably be explained by difficulties in identifying the right colony or overgrowth from neighbouring colonies rather than a poor specificity of the nucleotide probe. One isolate was found hippurate-negative, however, hippurate-negative *C. jejuni* strains do exist. In this study, *C. jejuni* was isolated from 4 of the 20 examined conventional pigs, which is a relatively high incidence compared to the Danish national surveillance programme, where mainly *C. coli* is found. Hence, this method could help to isolate different *Campylobacter* species in mixed populations and not only the most predominant species, which is likely to be chosen with the conventional method where a few colonies are randomly picked.

The colony blot hybridization can be performed either on all samples without testing of the *C. jejuni* status beforehand or on those samples only, where *C. jejuni* has been found (via pre-enrichment and rt-PCR). The later reduces the number of hybridizations if only few samples contains *C. jejuni* but in addition requires preparations of DNA and PCR. Another consideration, in case of a low level of campylobacter, the pre-enrichment may be necessary to obtain any colonies for hybridization.

The colony blot hybridization method is currently used to study the *Campylobacter* species distribution in 3x16 individual pigs in an outdoor production system (experimental setup) over time (7-14 week old pigs). Despite the expected higher occurrence of *C. jejuni* in outdoor pigs due to the exposure to wild life normally hosting *C. jejuni*, the results so far only indicate very few *C. jejuni* positive pigs, although *Campylobacter* were found in all pigs.

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O 45 Effect of an optimised pelleted diet on *Salmonella* prevalence and pig productivity

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Summary: The effect of an optimised, wheat based pelleted diet containing barley, sugar beet pulp and organic acids on *Salmonella* prevalence and pig productivity was investigated in two finisher herds. The optimised diet significantly reduced *Salmonella* seroprevalence compared to standard pelleted feed and meal feed. In contrast to previous studies, meal feed did not have a significant *Salmonella* reducing effect in this study. Meal diet but not the optimised diet had a significant negative effect on pig productivity, compared to the standard pelleted diet. Our results show that the optimised diet is a suitable alternative to wheat based pelleted feed or meal feed in reducing *Salmonella* prevalence in finisher pigs.

Keywords: feed intervention, barley, acid, coarse grinding, finisher pigs

Introduction: Several studies have shown that meal feed significantly reduces the prevalence of *Salmonella* in finishers, compared to pelleted feed. Meal feed, however, has the disadvantage of reducing pig performance, especially feed conversion. The aim of the present investigation was therefore to study the effect of an optimised, pelleted diet containing several *Salmonella* reducing properties (acid, barley, coarse grinding). The effect on *Salmonella* prevalence and pig productivity of this optimised diet was compared to standard wheat based diet fed as meal or as pellets.

Materials and Methods: The study was carried out in two swine herds with subclinical *Salmonella* infections. The study comprised 3 diet groups with approx. 600 growing/finishing pigs per group, divided into 43 replicates (23 and 20 replicates in each herd, respectively). **Group 1:** standard wheat based, pelleted diet; **group 2:** wheat based diet fed as meal; **group 3:** optimised wheat based pelleted diet containing barley (35%), sugar beet pulp (10%) and organic acid (0.6% formic acid + 0.6% lactic acid).

The effect of the 3 diets on *Salmonella* prevalence was measured by use of serology. Blood samples were collected randomly from 6 finisher pigs per pen in each replicated trial before transfer to slaughter. Blood samples were analysed using the Danish mix-ELISA with cut-off 20 OD%. In addition, average daily weight gain, feed intake, meat percentage, number of treatments due to disease, and mortality were registered. Based on these registrations and actual pig and feed prices, a production value (PV) was calculated.

Table 1. Effect of a *Salmonella* optimised diet on *Salmonella* prevalence in finisher pigs.

| Parameters | Diet | | |
|---|---------------|-----------|---|
| | Pelleted feed | Meal feed | <i>Salmonella</i> optimised pelleted feed |
| Herd A | | | |
| No. of pigs sampled | 138 | 138 | 138 |
| % pigs seropositive for <i>Salmonella</i> | 59 | 72 | 45 |
| Herd B | | | |
| No. of pigs sampled | 120 | 120 | 120 |
| % pigs seropositive for <i>Salmonella</i> | 50 | 37 | 21 |
| Total | | | |
| No. of pigs sampled | 258 | 258 | 258 |
| % pigs seropositive for <i>Salmonella</i> | 55 | 55 | 34 |

Results: Overall, a significantly lower *Salmonella* seroprevalence of 34% was found in pigs fed the optimised pelleted feed, compared to 55% for both standard pelleted feed and meal feed ($p < 0.01$). However, there was a marked difference between the two herds in seroprevalence level, particularly in the meal fed pigs (Table 1).

Overall, there was a significantly higher risk of detecting seropositive pigs in groups fed a standard pelleted diet (OR=2.5, $p=0.003$) or meal diet (OR=2.6, $p=0.002$), compared to pigs fed the optimised pelleted feed. Herd differences were observed, particularly regarding the meal fed groups, but these were not significant.

Due to technical errors, analysis of the productivity data is based on results from herd A only. The optimised pelleted diet resulted in a lower Production Value index, but this was not significant.

compared to standard pelleted feed. In contrast, productivity was significantly affected by the meal diet ($p < 0.05$), resulting in a poorer feed conversion compared to standard pelleted feed (Table 2).

Table 2. Effect of a *Salmonella* optimised diet on productivity of finisher pigs. Values with different subscripts differ at the 95% confidence interval.

| Parameters | Diet | | |
|------------------------|------------------|-----------------|------------------------------------|
| | Pelleted feed | Meal feed | Salmonella optimised pelleted feed |
| FUp/pig/day | 2.42 | 2.45 | 2.50 |
| Average daily gain (g) | 900 | 834 | 895 |
| Meat percent | 60.1 | 60.5 | 60.3 |
| Production value (EUR) | 108 ^a | 88 ^b | 102 ^a |
| Index | 100 | 82 | 95 |

Discussion and conclusion: Our results indicate that pelleted feed may reduce *Salmonella* prevalence in finishing pigs, when *Salmonella* reducing factors such as acid, barley and course grinding are included in the diet. In contrast to meal feed, the optimised diet did not significantly reduce pig performance but the production cost of the optimised pelleted diet is higher.

The observation that a wheat based meal diet failed to reduce *Salmonella* prevalence in finishers significantly compared to a standard pelleted diet, is in contrast to previous experiences. However, as Table 1 clearly shows the effect of meal diet differed quite remarkably between the two herds. The high seroprevalence in meal fed pigs in herd A may be due to the fact that this herd was placed in Level 3 of the *Salmonella* Control Program (indicating a high infection level in the herd) during most of the investigation period. The results in herd B are in accordance with previous studies, even though this herd also experienced a rise in seroprevalence during the study period.

Our results show that an optimised, wheat based pelleted diet containing barley, sugar beet pulp and organic acids is able to reduce *Salmonella* seroprevalence in finishers without significant negative effects on productivity. The optimised diet is therefore a suitable alternative to meal feed and standard pelleted feed. The results furthermore illustrate that feeding strategies alone are not sufficient to combat *Salmonella*.

O 46

Effect of feeding strategy on *Salmonella* in Danish sows and weaners

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Summary: The aim of this study was to investigate the effect of feeding strategy on *Salmonella* prevalence in sows and weaners. The owners of 228 sow herds were interviewed about their feeding strategy with regard to 1) use of home mixed meal feed contra pelleted feed, 2) heat-treated protein contra non-heat-treated protein (soy protein), 3) contents of high-fibre grain types (barley, oat and

sugar beet pellets) contra wheat, 4) use of organic acids in feed or drinking water, and 5) liquid/fermented feed contra dry feed. The answers were compared with the bacteriological and serological Salmonella data from each herd. No statistical significant effects were found of feeding strategy on Salmonella in these groups. Other factors like housing, management, cleaning and disinfection were not included in this study, and therefore their effect cannot be excluded.

Keywords: Salmonella, feeding strategy, barley, soy protein, organic acids.

Materials and methods: From January to June 2003, 228 Danish herd owners that had received a request for having their herds tested microbiologically according to the Danish Salmonella Control Program, were interviewed about feeding strategy in sows and weaners: home-mixed meal feed vs. pelleted feed, use of non-heat-treated soy protein vs. heat-treated protein in home-mixed feed, ratios of barley, wheat, oat, and sugar beet pellets, use of organic acid in feed and/or drinking water, and liquid feed vs. dry feed (the time of fermentation was not included in the query). The following age categories were included: Pregnant sows, lactating sows, and weaners. The weaners were typically fed 2 or 3 different types of feed from 7 to 30 kg of weight.

Using Fisher's Exact Test in SAS, the five feeding parameters and combinations hereof were tested against the results from the pen faecal samples. 42% of the tested herds were culture positive. The parameters were also tested against percent positive pen faecal samples. Furthermore, the five parameters were tested against the serological results from meat juice sampling in the 79 herds with finishers.

Results: The feeding strategy in the herds is shown in Table 1. 52% of the herds used organic acids for the weaners in drinking water or feed. The concentration in drinking water was typically 0,2% while the acid contents in feed typically were below 0,5% and often not available (not written on the declaration).

Table 1. Feed type use in the interviewed herds. # explained under "discussion."

| Feeding strategy | Sows | Weaners |
|---|------|---------|
| Home-mixed meal feed | 50% | 33% |
| Soy protein in home-mixed feed | 53% | 21% |
| 25% or 15% barley, oat or sugar beet pulp # | 70% | 78% |
| Organic acids | 0% | 52% |
| Liquid feed (partly fermented) | 21% | 21% |

Table 2 shows the type of feed for sows factors compared with the microbiological results from pen faecal samples. Some herds used more than one type of feed for the group. These are not included in the table.

Table 2. Correlation between sow feed and prevalence of Salmonella positive pen fecal samples.

| Type of sow feed | Number of positive herds (%) | Number of herds | P-value (Chisquare) |
|------------------|------------------------------|-----------------|---------------------|
| Pelleted | 49 (48 %) | 102 | 0.09 |
| Home-mixed | 36 (36 %) | 97 | |

No significant differences were found on 5% level, but a clear tendency was observed (see Table 2). Herds with positive microbiology or more than 40 percent positive meat juice samples included approx. 50% of the herds. When the five feeding parameters were tested against this group, there was no significant difference either. The prevalence of Salmonella was higher (not significantly)

among the herds using organic acids for the weaners. As expected there was a positive correlation between microbiology and serology. Thus, the chance of finding Salmonella in pen faecal samples was higher with increasing serology.

Discussion: The criteria for the query were based on research in the Danish swine production, primarily the Salmonella reducing effect of meal feed in finishers, and use of acids in water and feed for weaners, finishers and sows (Jørgensen et al., 2001, Kjorsgaard et al., 2001). There are two important biases in this investigation that make it difficult to show a clear effect. Firstly, only herds that have delivered pigs to finisher herds in level 2 or 3 are included. Kranker et al. (2001) demonstrated that buying pigs from infected sow herds is a main risk factor. This means that Salmonella-negative herds have less risk of being tested. The other important bias is that sow herds with a production of finishing pigs can have the request cancelled, if they can demonstrate, that their finishers are serologically negative. These herds are more likely to use protective management and feed factors, but are not included in this study.

Despite these results, we were able to demonstrate a tendency towards a protective effect of home-mixed meal. Among herds using organic acids in feed or drinking water for the weaners a higher, not significant Salmonella prevalence was found. This tendency might reflect the veterinarian's recommendation to start using acid in Salmonella-positive herds. The relatively low acid content in the feed and drinking water is another possible explanation. Factors like live-pig trading habits, continuing housing/all-in all-out, general management; cleaning and disinfection were not included in the query. These factors are known to play important roles concerning Salmonella reduction.

Conclusions: The results of this study confirm previous results that describe a larger complexity regarding Salmonella in sow herds compared to finisher herds. Use of the general recommendations for feed intervention against Salmonella in pigs did not result in clear-cut, reducing effects on Salmonella in sows and weaners. Other measures such as cleaning, disinfection and management may be more important.

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O 47 Feeding fermented liquid feed to the gestating sow can reduce pathogen challenge of the neonatal environment

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Summary: This study demonstrated that the coliform and *E. coli* challenge to the newborn piglet can be reduced by feeding sows fermented liquid feed (FLF). The faeces excreted by sows fed FLF

had significantly lower numbers of *E. coli* at parturition compared with sows fed non-fermented liquid feed (NFLF) and dry feed (DF). More importantly, significantly lower numbers of both coliforms and *E. coli* were found in the faeces of FLF-fed sows throughout the whole lactation period. The faecal *Lactobacillus*: *E. coli* ratio of piglets from FLF-fed mothers was significantly higher than for piglets born to DF-fed mothers. Higher *Lactobacillus*:*E. coli* ratio is usually associated with a bacterial flora that contributes to improved animal growth and performance.

Keywords: *E. coli*, coliforms, Lactobacilli, lactating sow, neonatal piglet

Introduction: One of the key environmental factors, which can have a big influence on newborn health and performance, is hygiene of the farrowing house. Gastrointestinal infections associated with *E. coli* represent a serious problem for neonatal pigs. It is known that the stress occasioned by movement into the farrowing house and parturition may lead to a decreased excretion of *Lactobacilli* and increased excretion of potential pathogens especially haemolytic *Escherichia coli* (Maclean & Thomas, 1974). The main aim of this study was to investigate the potential of fermented liquid feed to control the pathogen load within the piglet's environment by reducing the rapid multiplication of *E. coli* in sows associated with farrowing.

Materials and Methods: A study was conducted according to a randomized block design, with two replicates. Eighteen gilts (Large White x Landrace) were randomly allocated to one of the three dietary treatments namely: fermented liquid feed (FLF), non-fermented liquid feed (NFLF) and dry feed (DF) in pelleted form. *Lactobacillus salivarius*, of pig origin, was used as a starter culture for FLF. Feeding took place twice a day for a period of 2 weeks before farrowing date, and for 3 weeks after farrowing. The inoculated feed was fermented for 36 hours at 30 °C. Fresh faecal samples were collected from the rectum of each sow each week during the experimental period. Rectal swabs were taken from individual piglets 14 days post farrowing. Lactobacilli, coliforms and *E. coli* were analysed in each faecal sample by standard methods. A PCR-based identification method was used to monitor the faecal presence of the *Lactobacillus salivarius* strain used as a starter culture. Statistical analyses were undertaken using GLM-ANOVA.

Results: While the *Lactobacilli* population was not affected by dietary treatment, significant differences in coliform and *E. coli* populations were observed in the sow faecal samples taken at parturition as well as post farrowing (Table 1). The faeces excreted by sows fed FLF had significantly lower numbers of *E. coli* at parturition compared with sows fed NFLF ($P < 0.05$) and DF ($P < 0.0001$). These significantly lower numbers of *E. coli*, as well as coliforms in the faeces of FLF-fed sows, were maintained throughout the lactation.

Table 1. Microbial counts (\log_{10} cfu g^{-1} (dry weight)) of Coliforms, *E. coli* and *Lactobacilli* in the gilt's faeces 14, 7 days before farrowing (BF), at farrowing (F) and 7, 14 and 21 days post farrowing (PF). FLF-fermented liquid feed; NFLF-nonfermented liquid feed; DF-dry feed. ^{ab} Within columns, means with a common superscript are not statistically different.

| | Coliforms | | | | | |
|------|---------------------|-------------------|------------------|------------------|-------------------|-------------------|
| | 14BF | 7BF | F | 7PF | 14PF | 21PF |
| FLF | 5.8 | 5.8 | 6.2 | 5.1 ^b | 5.2 ^b | 5.1 ^b |
| NFLF | 5.9 | 5.7 | 6.4 | 6.5 ^a | 5.8 ^a | 6.0 ^a |
| DF | 5.9 | 6.2 | 6.6 | 6.3 ^a | 6.1 ^a | 6.0 ^a |
| | <i>E. coli</i> | | | | | |
| | 14BF | 7BF | F | 7PF | 14PF | 21PF |
| FLF | 5.2 | 5.5 | 5.0 ^b | 4.6 ^b | 5.0 ^b | 5.0 ^b |
| NFLF | 5.7 | 5.4 | 5.9 ^a | 5.8 ^a | 5.4 ^{ab} | 5.7 ^a |
| DF | 5.5 | 5.8 | 6.3 ^a | 5.9 ^a | 6.0 ^a | 5.9 ^a |
| | <i>Lactobacilli</i> | | | | | |
| | 14BF | 7BF | F | 7PF | 14PF | 21PF |
| FLF | 8.0 | 7.7 ^a | 7.4 | 7.3 | 7.6 ^{ab} | 8.0 ^a |
| NFLF | 8.1 | 6.7 ^b | 7.0 | 7.5 | 7.9 ^a | 7.9 ^{ab} |
| DF | 8.0 | 7.3 ^{ab} | 7.2 | 7.4 | 7.5 ^b | 7.6 ^b |

Table 2 *Lactobacillus* : coliform and *Lactobacillus*: *E. coli* ratio in the faeces of 2-week old piglets FLF-fermented liquid feed; NFLF-nonfermented liquid feed; DF-dry feed; ^{ab} within the rows, means with the same superscript are not significantly different.

| | FLF | NFLF | DF |
|------------------------------|-------------------|-------------------|--------------------|
| Lactobacilli: Coliforms | 0.33 ^a | 0.31 ^a | -0.19 ^b |
| Lactobacilli: <i>E. coli</i> | 0.42 ^a | 0.40 ^a | -0.17 ^b |

The faecal *Lactobacillus*: *E. coli* / coliform ratio of piglets from liquid-fed mothers was significantly higher ($P < 0.01$) than for piglets born to DF-fed mothers (Table 2). PCR method confirmed that the *L. salivarius* strain, used to ferment the feed, survived passage through the intestinal tract of sows and that it was present also in piglets' faeces on the 14th day of suckling.

Discussion: These results demonstrate that the coliform and *E. coli* challenge to the newborn piglet can be reduced by feeding sows fermented liquid feed. A similar beneficial effect of FLF on the microbial ecology of the pig gut was obtained in the study of Moran (2001). Multiple factors may account for this beneficial effect of FLF, which may act independently or synergistically. The low pH of the diet, the high numbers of lactobacilli and high concentration of lactic acid represent the most important characteristics of FLF in terms of its protective effect. The ability of LAB to inhibit the growth of various gram-negative bacteria, especially pathogenic *E. coli*, is well documented both *in vitro* (Jin et al., 2000) and *in vivo* (Muralidhara et al., 1977). Higher faecal *Lactobacillus*: Coliform / *E. coli* ratio is usually associated with bacterial flora that contributes to improved animal growth and performance (Muralidhara et al., 1977).

Conclusions: This study demonstrated that by appropriate nutritional regimes there is an opportunity to beneficially influence the sows' bacterial excretion, which could be reflected in more 'friendly' bacterial flora in the neonate GI tract. This approach would represent a very natural way of protecting piglets during this short but critical period after birth.

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O 48 A Randomised Controlled Trial To Reduce *Salmonella* Infection In Finisher Pigs

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Summary: Twenty-two finisher farms were randomly assigned to an intervention or a comparison group. The intervention group implemented a package of hygiene and biosecurity measures to

reduce *Salmonella* infection, measured by culture of pooled pen faecal samples and use of the meat juice enzyme-linked immunosorbent assay (MJ-ELISA). Data on hygiene and biosecurity practices were used to calculate compliance scores, which were significantly greater on intervention farms. *Salmonella* was isolated from 38% (95% confidence interval [ci] 22% - 53%) of pens on intervention farms and 42% (95% ci 27% - 58%) of pens on comparison farms. The prevalence of MJ-ELISA positive pigs on intervention farms was 40% (95% ci 26% - 58%) and 58% (95% ci 41% - 75%) on comparison farms. These differences were not statistically significant. The power of this study was reduced by a strong farm effect. The prevalence of infection amongst introduced pigs at the start of the finisher cycle had a significant impact upon overall pen prevalence.

Keywords: Intervention; UK; epidemiology; hygiene; biosecurity

Materials and Methods: All farms that were contracted to raise finisher pigs for one integrated company were invited to participate in this study and 22 farms volunteered. These farms followed a strict all in/all out policy and used the same feed and management regimes. At an initial meeting, a package of feasible measures intended to reduce *Salmonella* infection was agreed and farmers were then allocated to either an intervention or a comparison group by drawing names from a hat. Intervention measures were intended to improve between-batch hygiene, limit the risk of introducing *Salmonella* and reduce the risk of transmission during production. A total of 24 different measures were described and a compliance score was calculated for each of the 22 farms, according to the number of these measures that were actually undertaken. Every farm completed an initial questionnaire and a weekly recording sheet documenting compliance. Pooled faecal samples (25g) were collected from a random selection of pens within 24 - 72 hours of the arrival of the pigs and these pens were then sampled at approximately monthly intervals. Samples were pre-enriched in buffered peptone water and selectively enriched in Diassalm agar plates. Samples from this were inoculated onto Rambach agar and suspect *Salmonella* colonies were subjected to a slide agglutination test using a range of typing sera and to the minimum phenotypic criteria for identification to *Salmonella* species. A subculture of each confirmed *Salmonella* isolate was submitted for full serotyping and phage typing, where applicable (Davies et al 2001). Meat samples were obtained from a random sample of 40 pigs from each unit shortly after slaughter. The meat juice was tested using an enzyme-linked immunosorbent assay (MJ-ELISA) for antibodies to group B and C1 *Salmonella* serotype lipopolysaccharide. Samples were tested in duplicate by the Guildhay VetSign *Salmonella* ELISA Kit, according to the manufacturer's instructions. During testing, field samples of strong and weak positive sera and meat juice were run alongside the tests, as well as the kit controls. Plate results were accepted when all controls met the expected results. A cut-off value of 0.25 (sample:positive ratio) was used to classify samples as positive or negative. All data were stored in MS ACCESS and then analysed using Stata v8 software.

Results: Intervention farms showed significantly improved hygiene and biosecurity practices with a mean compliance score of 15.2 (95% ci 13.8 - 16.5) compared to 7.7 (95% ci 4.6 - 10.8) for comparison farms (t-test; $p < 0.001$). Particular intervention measures that were followed more assiduously included - use of detergents and disinfectants when cleaning pens and feed hoppers; disinfection of muck heap sites and cleaning and disinfecting tractors, scrapers and other equipment regularly. However, the mean number of sources of weaners used to restock the farms was 2 (95% ci 1 - 3) for intervention farms and 3 (95% ci 1 - 5) for comparison farms - this was not significantly different (t-test; $p = 0.362$). The mean time from the last pig of the previous batch leaving a farm and the first pig of the trial batch arriving was 26 days (95% ci 16.4 - 35.2) on comparison farms and 33 days (95% ci 15.7 - 49.7) on intervention farms, but this difference was not significant (t-test; $p = 0.380$). Pens were first sampled within 24-72 hours of the arrival of the first pigs to detect introduction of infection. There was no significant difference in the prevalence of infected pens at this time (28% for comparison farms and 30% for intervention farms; 95% ci's 18% - 38% and 17% - 42% respectively). No significant differences were detected in herd performance (daily liveweight gain, days to slaughter, mortality or feed conversion

efficiency). By the end of the trial, the mean prevalence of pens from which *Salmonella* was isolated at least once was 42% (95% ci 27% - 58%) for comparison farms and 38% (95% ci 22% - 53%) for intervention farms. The prevalence of MJ ELISA positive pigs was 40% (95% ci 26% - 58%) on intervention farms and 58% (95% ci 41% - 75%) on comparison farms; this difference was not statistically significant (t-test; $p=0.118$). It was noted that one farm in the intervention group had no *Salmonella* isolated from any pen during the trial and only one of the 40 pigs tested had a positive MJ ELISA result. The data were used to investigate the impact of the prevalence of infected pens at the first sampling visit upon the outcome of the trial. There was a significant relationship between the prevalence of infected pens at the start of the trial and of the prevalence of pens that were ever infected ($p<0.001$). This is unsurprising, since across the whole trial 324 out of a total of 787 pen samples were positive and 242 of these occurred at the first visit. A pen from which *Salmonella* had been isolated on the first visit was nearly twice as likely to have *Salmonella* isolated on a subsequent occasion, compared to a pen that was negative on the first visit (odds ratio = 1.9; 95% ci 1.2 - 2.9). No significant association was detected between the prevalence of infected pens at the first visit and the prevalence of MJ ELISA positive pigs.

Discussion: This trial was conducted on a group of farms that already had a good awareness of biosecurity and hygiene. The intervention group achieved a commendable increase in their activities, but this was not rewarded by a significant decrease in either the prevalence of infected pens or of MJ ELISA positive pigs. However, this trial was relatively small and the results are compatible with a potentially important reduction in *Salmonella*. Thus, a more extensive trial is justified. A major factor that may confound the beneficial effects of the intervention was the level of infection introduced by weaners.

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O 49 Selection of finishing pig herds with a low *Salmonella* prevalence for logistic slaughtering.

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Summary: The aim of this study was to select 50 herds with a low *Salmonella*-contamination rate. Per herd 40 blood samples were collected which had to be negative for antibodies against *Salmonella*. Infection of the pigs at the abattoir was measured by culturing tonsils for *Salmonella*. The results showed that not enough herds could be selected when the original criteria were applied. Less strict criteria had to be applied. We conclude from this trial that it is possible to select herds with a lower *Salmonella*-prevalence resulting in a lower introduction of *Salmonella* into the abattoir. Strict criteria must be applied to select herds with a minimal risk of being *Salmonella*-positive. To select *Salmonella*-free herds bacteriological examinations are necessary in addition to serological screening. Prolonged monitoring with a high frequency is necessary. We conclude that the effectiveness of the cleaning and disinfection protocols for transport vehicles should be improved.

Keywords: Elisa, Bacteriology, carcass contamination, abattoir, transport

Introduction: A trial study was set up with the aim to demonstrate that it is possible to produce pig carcasses with a very low *Salmonella*-contamination rate under practical abattoir conditions. The trial consisted of two phases, in the first phase the aim was to select 50 herds with a low *Salmonella* prevalence and in the second phase the hypothesis was tested that a system of logistic slaughter can produce pig carcasses with a low *Salmonella* contamination rate. 219 Herds were pre-selected from the regular suppliers of the abattoir to participate in phase 1. To select herds, blood samples were taken at the abattoir and tested in a *Salmonella*-mix-ELISA (Idexx). To demonstrate a prevalence of less than 5%, with a level of confidence of 95%, 40 blood samples have to be taken (van der Wolf et al., 2001) which all must have a negative test result. This paper describes the results of the herd selection process, Mrs. Swanenburg will present the results of the second phase.

Materials and Methods: To avoid the influence of clustering at least 4 batches of pigs of each pre-selected herd had to be tested with a maximum of 10 blood samples collected per batch. Blood samples were tested in the Dutch *Salmonella* mix ELISA (Idexx) using a cut-off OD% > 10%. Lorries that delivered a batch of pigs to the abattoir were sampled directly after the pigs were unloaded by taking five faeces droppings (total 25 gr) from the floor of the lorry. From herds that were selected for the logistic slaughter trial and from which one lorry sample was positive, four pooled faecal samples were collected during a visit to the herd. Each pooled faecal sample represented one compartment and consisted of at least one fresh dropping from the floor of every pen (8 – 10) in the compartment. Culturing of faeces was done to demonstrate the presence of *Salmonella* (qualitative), using standard microbiological culturing including non-selective pre-enrichment (BPW), selective enrichment (RV or TTB) and sero-typing. Final sero- and phagotyping was done at the *Salmonella*-NRL. Statistical analysis was done with the Fisher exact test in the program Statistix for Windows 7.0. P-values of less than 0.05 were considered to be significant.

Results: A total of 7646 blood samples were collected and tested (Table 1). *Salmonella* was isolated from 49 (45%) of the total of 109 lorry faecal samples. Three herds were excluded from the trial because both lorry faecal samples were positive. Combining the results of the serological and faecal investigations resulted in the selection of 24 herds to participate in the trial. However, since the aim was to select 50 herds less strict criteria were used to select additional herds (Table 2). During the start of phase 2 twenty herds were visited during which visit pen faecal samples were collected. *Salmonella* was isolated in 9 out of 20 herds (45%). In one herd the serology was also positive. Of these 20 herds 10 were selected based on the original selection criteria. Of these 10 herds 3 tested positive. Of the 9 remaining herds (one was serologically and bacteriologically positive) which were selected based on the less strict criteria 6 herds tested positive. This difference is large but not statistically significant (Statistix 7.0, Fisher Exact test, upper tail, P=0.08).

Discussion: All stages of the production process - herd, transport and lairage - have to contribute to a low level of contamination of slaughter pigs with *Salmonella* because a laps in hygiene in any stage can result in contaminated pigs entering the abattoir. Due to the fact that not enough herds could be selected using the original criteria and less strict criteria had to be applied, the risk of herds not being *Salmonella*-free increased. This was confirmed by the observation that of the herds which were selected with the less strict criteria a larger number had *Salmonella*-positive pen faecal samples than the herds that were selected with the strict criteria. We conclude from this observation that the strict criteria are necessary to select herds with a minimum of risk of being *Salmonella*-infected. However, also the herds that were selected based on the original criteria were not completely *Salmonella*-free. There are three explanations for this. First, the assumption in the samples size (prevalence <5%), secondly, the level of confidence of 95%, and thirdly, the sensitivity of the *Salmonella* mix-ELISA test is not 100% but only 85 to 90%. We conclude from this that bacteriological investigations could be useful in addition to serological investigations to select *Salmonella*-free herds. An additional problem encountered is that the *Salmonella*-status of herd can change with time. We conclude that repeated sampling is crucial for a good assessment of the *Salmonella*-

status. Bacteriology of the faecal samples from the lorries shows that they are frequently contaminated with *Salmonella*. Our data suggest that the contamination could not always be attributed to the just unloaded pigs. In six herds blood and pen faecal samples were all negative while one or more corresponding samples of the lorries were positive. The *Salmonella* serotypes found on the lorries would, if they had been present in the herd, have given a clear serological response in the *Salmonella* mix-ELISA test. Therefore we conclude that the effectiveness of the cleaning and disinfection protocols should be improved.

Table 1. Results of the serological testing for antibodies against *Salmonella* (cut-off%=10¹) in blood samples from 219 finishing pig herds.

| Percentage positive samples | Number of herds per category |
|-----------------------------|------------------------------|
| 0 | 39 (17.8%) |
| >0-25% ² | 118 (53.9%) |
| >25-50% | 41 (18.7%) |
| >50-75% | 13 (5.9%) |
| >75% | 8 (3.6%) |

¹ results with an OD%-value > 10 are considered positive.

² percentage positive samples of the total number of samples per herd.

Table 2. Selection criteria for participation in the trial and number of selected herds.

| Criteria | Number of herds |
|---|-----------------|
| 40 or more blood samples, all negative, maximum 1 positive lorry faecal sample ^A | 24 |
| 60 or more blood samples, maximum 1 positive, maximum 1 positive lorry faecal sample ^B | 17 |
| 20 or more blood samples, all negative, no positive lorry faecal samples ^B | 9 |
| 45 blood samples, 1 positive, no positive lorry faecal samples ^B | 2 |
| Total | 52 |

A: initial criterion, B: Criterion added later on.

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O 50 Cost-effectiveness of *Salmonella* control in the pork chain using maximum acceptable prevalence levels

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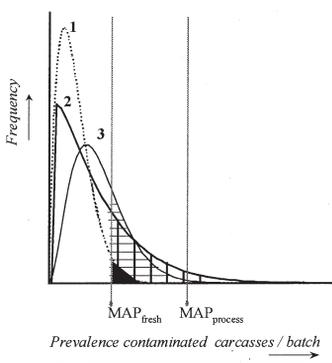
Summary: To motivate stages in the pork chain to control *Salmonella*, Maximum Acceptable Prevalence (MAP) levels can be defined whereas exceeding these levels results in a penalty. A simulation model was used to determine the effect of different control strategies on the prevalence of contaminated carcasses. Not implementing any control strategy in the chain is not the cheapest option in case the revenues per batch of carcasses depend on the prevalence. Hence segmentation of batches of carcasses based on their prevalence can be useful to increase food safety.

Keywords: food safety, economic evaluation

Introduction: To control the food safety of pork with respect to *Salmonella* contamination, multiple stages of the supply chain have to be involved (Berends et al. 1998). The basic end-product of the pork chain is

a chilled carcass at the slaughterhouse. Therefore, the first focus should be on the reduction of prevalence of carcasses contaminated with *Salmonella* at the end of the slaughterline. To motivate stages in the pork chain to control *Salmonella*, Maximum Acceptable Prevalence (MAP) levels can be defined and exceeding of these levels results in a penalty. This paper presents briefly the preliminary design of a detailed research on the feasibility of using Maximum Acceptable Prevalence levels as a control strategy.

Materials and Methods: A batch of carcasses with a high prevalence causes a higher risk for food borne diseases when the batch is sold for fresh meat products than when the batch is processed to cooked or fermented meat products. Consequently, two MAP values can be distinguished: MAP_{fresh} for fresh pork products and MAP_{process} for production of processed pork products. The MAP_{fresh} should be lower or at least equal to MAP_{process}. Batches that exceed the MAP_{process} are not suitable for regular processing and are used for low-grade products or pet food. Figure 1 presents three hypothetical distribution functions for the prevalence of contaminated carcasses per batch. Each distribution represents the frequency of the prevalence of batches contaminated carcasses of one farm or firm.



In distribution 1, only a few batches exceed the MAP_{fresh} (black surface). These batches do not exceed MAP_{process} and are suitable for regular processing. Distribution 2 shows much more batches that exceed MAP_{fresh} (vertically hatched surface) and several batches that exceed MAP_{process}. Distribution 3 has almost no batches that exceed MAP_{process}, but relatively many batches that exceed MAP_{fresh} (horizontally hatched surface).

Figure 1 Three hypothetical distributions of the prevalence of contaminated carcasses per batch per farm or firm

When both MAP_{fresh} and MAP_{process} are determined, three segments of carcasses can be distinguished: batches for fresh meat products, batches for processed products and batches for low-grade products. A testing procedure is required to assign batches to a segment. To reduce the prevalence of *Salmonella*, the finishing and slaughtering stage can choose among three strategies: D (default, no control), P (preventive control) and T (total control). Total control includes, besides the preventive measures, additional measures that are implemented temporarily when the prevalence exceeds a predefined threshold. To be able to know when the additional measures should be carried out, a serological test was included for the finishing stage and a bacteriological test for the slaughtering stage. The costs for both tests were €2 per pig or carcass and all individuals were tested

Table 1 Characteristics and costs of three control strategies for two stages

| Control strategy | Preventive measures (carried out continuously) | | Additional measures (temporary after exceeding predefined prevalence, so costs are variable depending on the prevalence over time) | |
|------------------|--|-----------------------|--|---------------------|
| | Finishing | Slaughtering | Finishing | Slaughtering |
| Default | No | No | No | No |
| Preventive | Yes (€ 2.8 /100 pigs/d) | Yes (€ 0.9 / carcass) | No | No |
| Total | Yes | Yes | Yes (€ 0.6/100pigs/d) | Yes (€ 0.4/carcass) |

The maximum revenues in the chain are achieved in case there are no costs and all batches are in the best *Salmonella* segment, yielding the highest revenues. However, this is not realistic since there are costs for control and for testing and not all batches are in the best segment. The costs are calculated as additional costs compared to the default situation where no control measures are

implemented. In case batches of carcasses are in the segment of processed or low-grade meat products, the revenues are reduced. These reductions of revenues can be seen as revenues forgone.

The stages finishing and slaughtering are included in this study. With a simulation model (described in Van der Gaag et al., 2003), scenario studies were carried out whereas the two stages followed different control strategies to reduce the prevalence of *Salmonella*. The output of the model was the contamination prevalence per batch of 100 carcasses.

Results and discussion:

Table 2 Costs, prevalence segmentation, revenues and cost-effectiveness of a typical scenario as an example (C_c and C_t are costs for respectively control and testing, B_f is the fraction of batches with prevalence $< MAP_{fresh}$, B_r is the fraction of batches with prevalence $> MAP_{process}$ and B_p is $1-B_f-B_r$)

| Control Strategy* | C_c (€) | C_t (€) | B_f (fraction) | B_p (fraction) | B_r (fraction) | Revenues forgone | Average prevalence long-term (%) | Total costs (€ per pig) |
|-------------------|--------------|--------------|---------------------|---------------------|---------------------|------------------|----------------------------------|----------------------------|
| D&D | 0.00 | 0.20 | 0.204 | 0.760 | 0.036 | 2.83 | 12.24 | 3.03 |
| P&D | 2.80 | 0.20 | 0.570 | 0.430 | 0.000 | 1.29 | 5.55 | 4.29 |
| T&D | 2.80 | 2.25 | 0.704 | 0.294 | 0.002 | 0.92 | 4.38 | 5.97 |
| D&P | 0.94 | 0.20 | 0.886 | 0.114 | 0.000 | 0.34 | 2.75 | 1.48 |
| P&P | 3.74 | 0.20 | 0.988 | 0.012 | 0.000 | 0.04 | 1.29 | 3.98 |
| T&P | 3.74 | 2.34 | 0.989 | 0.011 | 0.000 | 0.03 | 0.97 | 6.11 |
| D&T | 0.94 | 2.17 | 0.929 | 0.071 | 0.000 | 0.21 | 1.75 | 3.32 |
| P&T | 3.74 | 2.03 | 0.988 | 0.012 | 0.000 | 0.04 | 1.17 | 5.81 |
| T&T | 3.74 | 4.24 | 0.993 | 0.007 | 0.000 | 0.02 | 0.90 | 8.00 |

* strategy of finishing and of slaughtering stage, i.e. D&D indicates that both stages follow Default strategy ($MAP_{fresh} = 5\%$, $MAP_{process} = 25\%$)

* strategy of finishing and of slaughtering stage, i.e. D&D indicates that both stages follow Default strategy ($MAP_{fresh} = 5\%$, $MAP_{process} = 25\%$)

The B_f , B_p and B_r depend on the distribution of the prevalence of batches (calculated by the epidemiological model) and the MAP_{fresh} and $MAP_{process}$. In the chain control strategy D&D, the percentage of batches with a prevalence below MAP_{fresh} is 20.4% (B_f D&D) and 3.6% (B_r D&D) of the batches exceed the $MAP_{process}$. The revenues forgone are the decrease in revenues per carcass due to the fact that some batches are not in the best segment and thus have lower revenues. The Total costs per strategy are the control costs ($C_p + C_t$) + revenues forgone.

Conclusions:

- Total control in finishing and slaughtering stage results in the lowest average prevalence (0.9%) but is also most expensive (total costs are _ 8.00 / pig)
- Not implementing any control strategy is not the cheapest option in case the revenues per batch depend on the prevalence (of course depending on the levels of the MAP's and the reduction in revenues). Hence segmentation of batches of carcasses based on their prevalence can be useful to increase food safety.
- Investments in control measures do not always result in a reduction of the prevalence.
- Controlling *Salmonella* only in the slaughtering stage is more effective than only controlling *Salmonella* in the finishing stage.

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Control of Salmonella at pig finishing farms with a farm decision tree

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Summary: To decrease the prevalence of Salmonella in the Dutch pork chain a management tool is developed based on the HACCP methodology (Hazard Analysis of Critical Control Points) and tested at three pig farms on effectiveness and practical feasibility. A so called HACCP team defined Critical Control Points (CCP) and general measures. For all identified CCP's corrective actions are determined. Based on the obtained knowledge eight decision trees are designed and applied at three pig farms during eight months. The finishing pigs were tested bacteriologically and serologically every 2-3 month on *Salmonella typhimurium*. At one farm the number of positive samples declined over time. The other two farms showed a low prevalence during the entire trial period, even though all farms had a high prevalence in august 2001. It can be concluded that the decision trees are useful to determine weak points and to advice specific control measures to prevent or reduce Salmonella in pig farms.

Keywords: Hazards; HACCP methodology; weak points; farm specific measures.

Introduction: The Dutch Product Boards for Livestock and Meat is designing a Salmonella Control Program to reduce the number of cases of human salmonellosis. The focus of the program is on slaughterhouses and pig farms. The Product Boards asked the Applied Research of the Animal Sciences Group to develop and test a management tool to reduce the Salmonella prevalence at finishing farms.

Materials and Methods: To develop and test the tool, three steps were necessary: 1) identification of hazards, measures and corrective actions by means of the HACCP methodology. 2) Design of the tool and 3) Testing the tool by application at three pig farms.

Step 1) Hazards are identified by means of the HACCP methodology (FAO/WHO Codex Alimentarius Commission, 1993). However, four adjustments to the HACCP methodology were necessary: 1) instead of eradication of the hazard (demand of HACCP), reducing the hazard was accepted 2) the seriousness of the hazard was defined as the number of pigs at the farm that might become infected due to the hazard (instead of the seriousness of the hazard of the end product for humans) 3) A Critical Control Point (CCP) in this research did not have to meet the demand that the hazard in a later step of the process must not be eliminated and 4) in the hazard identification a distinction was made between the introduction and spread of Salmonella at pig farms. A HACCP team consisting of Salmonella experts and a farmer carried out the risk analysis for the introduction and spread of Salmonella at pig finishing farms. For each step in the process of pig production, hazards are identified.

Step 2) With the results of step 1, a management tool had to be designed that is user-friendly, effective, simple, cover all farm processes and completing it should not take too much time. The aim was a tool that determines at each kind of farm the specific control measures that can reduce the introduction and spread of Salmonella. Step 2 resulted in the development of eight decision trees with all hazards and control measures.

Step 3) The effectiveness and the practical feasibility of the decision trees were determined by applying a number of the specific control measures during eight months at three pig farms with

finishing pigs. It was known that these farms had a high *Salmonella* prevalence in August 2001. The finishing pigs were tested bacteriologically and serologically on *Salmonella typhimurium* every 2-3 months from May 2002 until December 2002. The faecal samples were tested qualitatively. The blood samples were tested with a mixed Elisa (positive when OD>10%). To get more insight in the opinion of the farmers, the farmers completed at the end of the trial period an evaluation form.

Results: Step 1) Three hazards were identified as CCP and 34 hazards were identified as a 'point of attention' (a point of attention is a hazard that can be reduced by implementing general control measures). The three CCP's, their limits, methods and frequencies of monitoring and their corrective actions are shown in table 1.

Table 1. Critical control points, their limits, methods and frequencies of monitoring and their corrective actions to prevent the introduction and spread of *Salmonella* at pig finishing farms

| CCP | Limits and tolerances | Method of monitoring | Frequency of monitoring | Corrective actions |
|--------------------|--|--|----------------------------------|--|
| Water from well | standards of the Animal Health Service** | Sampling according procedures of the Animal Health Service** | Twice a year | 1) according to advice of Animal Health Services or Veterinarian 2) acidify drinking water (3,5 < pH < 4,2) |
| Liquid by products | pH < 4 | Test pH at production level and at animal level | Every feeding | Adding acids |
| Piglets* | Salmonella negative piglets (bacteriologically tested) | Declared Salmonella free | Before every purchase of piglets | No purchase |

*This CCP can not be put into practice yet

** <http://portal.agroweb.nl> →GD

Step 2) To fulfil the requirements eight decision trees were designed covering all farm processes: 'supply of piglets', 'use of drinking water', 'use of pelleted feed', 'use of cereals', 'use of roughage', 'use of liquid by products', 'hygiene management' and 'daily management'. After completing the decision trees, the farm specific control measures that should be implemented to reduce introduction and spread of *Salmonella* are determined.

Step 3) At the first sampling period, only one farm had multiple positive samples. At this farm the number of positive samples declined over time. At the other two farms a very limited number of positive samples were found during the entire trial period.

Although the amount of time necessary to complete the decision trees was long (1-2 hours), the pig farmers indicated that the decision trees are user-friendly, complete and recommendable to other pig farmers.

Discussion, conclusions and recommendations: The results of the farm with a high prevalence at the start of the trial period seems promising. However, it is not possible to determine the effectiveness

of the advised control measures on the Salmonella prevalence at the farm based on this research due to the limited number of farms. The adjusted HACCP methodology is useful to determine weak points and the control measures in a structured way for, in this case, reducing the risk of introduction and spread of Salmonella at a pig finishing farm. The decision trees are a useful tool to specify the farm specific hazards and control measures.

The maximum benefit of the decision trees will probably be achieved when the decision trees are completed together with an advisor.

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MIXED CULTURE OF COMMENSAL BACTERIA REDUCES *E. COLI* IN NURSERY PIGS

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Summary: The purpose of the present study was to use field trials to evaluate the efficacy of a porcine-derived, defined culture (RPCF) of commensal bacteria for prevention of clinical disease from enterotoxigenic strains of *Escherichia coli* in weaned pigs. Neonates (< 24 h old) were orally administered RPCF and were monitored throughout the post-weaning nursery period on five geographically separated farms. The farms had a history of high mortality from F-18 strains of *E. coli*. RPCF-treated pigs had reduced mortality, morbidity, and medication costs from *E. coli* compared to untreated pigs. Although experimental, RPCF may become an effective control procedure for enterotoxigenic *E. coli*.

Keywords: enterotoxigenic, mortality, field trials, RPCF, medication costs

Introduction: *Escherichia coli* has been described as the major cause of neonatal and weaned pig diarrhea and death in pigs (Bertschinger *et al.*, 1992). Mortality and loss of productivity from edema disease cost the U.S. swine industry millions of dollars annually. The treatment of choice for an outbreak of enterotoxigenic *E. coli* has been antibiotics; however, due to antibiotic resistance, new control methods need to be explored. There appears to be global interest in the use of probiotics and competitive exclusion cultures as alternatives to antibiotics. The theory of competitive exclusion cultures works on the premise that when an animal is born, the intestinal tract is a sterile environment with none of the microflora found in healthy adults of that species. The absence of the normal microflora in the neonate predisposes it for colonization by enteropathogens. If adult microflora is administered to neonates, the gut will be colonized by commensal bacteria earlier than what would occur naturally and the neonate will be more resistant to colonization by enteropathogens (Nurmi and Rantala, 1973; Lloyd *et al.*, 1977). Some mechanisms by which commensal bacteria block colonization by pathogens include competition for nutrients, occupation of receptor and attachment sites, and production of bactericidal compounds. Our laboratory developed a defined, porcine-derived, mixed culture of commensal bacteria designated RPCF. *In vitro*, RPCF has prevented colonization by *Salmonella* and *E. coli* (Harvey *et al.*, 2002). In laboratory challenge studies with enterotoxigenic *E. coli*, RPCF-treated pigs had reduced mortality, decreased shedding, and decreased gut concentrations of *E. coli* compared to controls (Genovese *et al.*, 2000, 2001). The objective of the

present study was to evaluate the efficacy of RPCF to prevent or reduce disease associated with enterotoxigenic strains of *E. coli* in nursery-age pigs in commercial operations.

+Materials and Methods: Five farms (four nursery and one wean-to-finish) from various geographic regions of the U.S. that had been diagnosed with enterotoxigenic (F-18 strain) *E. coli* disease were selected for participation in these trials. Each farm had a history of high mortality from F-18 strain. Nine sow units supplied weaned pigs to these farms. Piglets were orally dosed with 10^8 colony-forming-units of RPCF within 24 h of birth, and performance records were measured from birth throughout the nursery period. Measurements included weaning weights and weaning mortality at the sow farm and average daily weight gain, feed consumption, feed efficiency, mortality and culls, and medication costs in the nurseries. A total of 34,676 pigs were included on trials and 20,217 piglets were treated with RPCF.

Results and Discussion: Disease associated with *E. coli* was reduced on each of the five farms. The most obvious improvement seen with RPCF treatments was decreased mortality and culls (Table 1). These results are similar to those seen in laboratory studies in which RPCF-treated piglets had reduced mortality, shedding, reduced organ invasion, and decreased GI tract concentrations when challenged with a 987p strain of *E. coli* (Genovese *et al.*, 2000, 2001). In the present study, cull losses were combined with mortality losses because the authors felt this was a more objective measure of morbidity and overall health of the herds. When mortality alone was measured, most of the RPCF-treated groups were at 1 % or less (data not shown). Decreased medication costs were calculated on four of the five farms. When projected to an annual basis, the medication cost savings were: Farm 1 = 1048 (\$), Farm 3 = 18,765 (\$), Farm 4 = 2970 (\$), and Farm 5 = 7560 (\$). The cost benefits to the producers from mortality reductions varied depending upon the size of the farm, but averaged 22,196 (\$) per farm (Table 1). Cost benefits were calculated thusly: Mortality percentage difference X annual number of pigs grown X 50 (\$) per pig = annual value; Medication cost difference per pig X annual number of pigs grown = annual value. On an annual basis, Farm 1 produced 2600 pigs, Farm 2 produced 8000 pigs, and Farms 3, 4, and 5 produced 27,000 pigs each. There were no consistent differences observed between treatments for weaning weights or weaning mortality or for feed consumption, feed efficiency, or average daily gain. The results from these trials demonstrate that RPCF is able to reduce mortality and morbidity and reduce medication costs associated with field challenge from F-18 strains of *E. coli*. Although RPCF is not commercially available at present, once marketed it could become an alternative to antibiotics in the control of enterotoxigenic *E. coli*.

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Table 1. *RPCF improves profitability on E. coli problem farms*

| Farm Identification U.S.) | Number Pigs on Test | Mortality difference (%) | Annual Value to Producer (\$) |
|---------------------------|---------------------|--------------------------|-------------------------------|
| 1 | 2619 | 4.80 | 6240 |
| 2 | 1000 | 5.00 | 20,000 |
| 3 | 13,644 | 6.20 | 66,960 |
| 4 | 11,218 | 0.80 | 8600 |
| 5 | 6195 | 0.85 | 9180 |

Trichinae Certification in the United States Pork Industry

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Summary: We report here on progress in the Trichinae Certification pilot program. This program uses an on-farm auditing system to document good production practices (GPPs) for swine relative to the risk of exposure to *Trichinella spiralis*. The pilot phase of this program continues while regulations to establish it as an official USDA program are being developed. Launch of the Trichinae Certification Program in the United States is expected to take place when regulations are finalized within the next year. The *Trichinella* certification program establishes a process for ensuring the quality and safety of animal-derived food products from the farm through slaughter.

Keywords: Food Safety, *Trichinella spiralis*, Pork, Trichinellosis, Preharvest Pork Safety.

Introduction: Control of *Trichinella* infection in U.S. pork has traditionally been accomplished by inspection of individual carcasses at slaughter or by post-slaughter processing to inactivate parasites. Declines in prevalence of this parasite in domestic swine during the last thirty years, coupled with improvements in pork production systems, allows pork safety, relative to *Trichinella* infection, to be documented at the farm level. We report here on progress in the Trichinae Certification pilot program.

Materials and Methods: Knowledge of risk factors for exposure of swine to *Trichinella spiralis* were used to develop an objective audit that could be applied to pork production sites. In a pilot study, 359 production site audits were performed by trained veterinary practitioners. Verification testing of swine raised on audited sites was subsequently performed using an ELISA test.

Results: The production site audit includes an assessment of farm management, bio-security, feed and feed storage, rodent control programs, and general hygiene. In pilot studies, objective measures of management practices were obtained through a review of production records and a site inspection. Of the 359 production site audits, 342 audits (95.3%) indicated adherence to management practices that met program standards and these sites were granted either entry into the program, or program certification. These sites will be audited regularly on a schedule established for the Trichinae Certification Program. Those sites that were audited and did not meet program standards for management practices did not gain entry into the program. Verification testing of swine from audited

production sites is ongoing at the slaughter plant. To date, random verification testing of swine from sites in the pilot program has resulted in only negative results for *Trichinella* infection.

Discussion: *Trichinella spiralis* is a parasitic nematode affecting animals and man. The disease caused by this parasite, trichinellosis, is acquired by consuming encysted larvae of *Trichinella spiralis* in the muscle tissue of an infected animal. Consumption of undercooked pork has traditionally been a common source of trichinellosis in humans worldwide. In the U.S. the prevalence of this organism in pigs has dropped sharply due to changes in swine management practices within the U.S. pork industry. In 1900, greater than 2.5% of the pigs tested were found to be infected with *Trichinella*. The infection prevalence declined to 0.95% in the 1930's, 0.63% in 1952, 0.16% in 1965, and 0.12% in 1970. The USDA National Animal Health Monitoring System's National Swine Survey in 1995 showed an infection rate of 0.013% (Gamble & Bush 1998). The same survey in 2000 demonstrated that the infection rate in U.S. swine had fallen to 0.007% (Bush 2002).

In the mid 1980s the convergence of three factors provided a powerful rationale for the development of industry supported programs to improve food safety in the U.S. First, the prevalence of *Trichinella* in U.S. swine had reached such a low level that disease free status could be envisioned. Second, there was recognition by U.S. pork industry leaders that international markets were closed to U.S. producers and U.S. pork products because of the now inaccurate perception that U.S. produced pork had a comparatively high risk of harboring *Trichinella*. Finally, the development of a rapid, ELISA-based diagnostic test provided a relatively inexpensive tool, which could be utilized in a control program.

The U.S. Trichinae Certification Program is a developing USDA program based on scientific knowledge of the epidemiology of *Trichinella spiralis* and numerous studies demonstrating how specific good production practices (GPPs) can prevent exposure of pigs to this zoonotic parasite. This program is a model program for on-farm assurance of product safety. The International Commission on Trichinellosis in their publication, Recommendations on Methods for the Control of *Trichinella* in Domestic and Wild Animals Intended for Human Consumption, states that, "Modern swine production systems reduce or eliminate risks of swine infection with *Trichinella* and testing of individual animals raised under these conditions could be eliminated." (Gamble et al., 2000). This publication continues with details of the requirements of such production systems. The Trichinae Certification Program meets these standards in all respects.

The U.S. Trichinae Certification Program is regulated by the U.S. Department of Agriculture (USDA). Collaborative efforts between the USDA's Animal and Plant Health Inspection Service (APHIS), the Food Safety Inspection Service (FSIS), and the Agricultural Marketing Service (AMS) assure that certified pork production sites manage and produce pigs according to the requirements of the program's GPPs and verify the identity of pork from the certified production site through slaughter and processing.

Conclusions: The described Trichinae Certification Program establishes a process for ensuring the quality and safety of animal-derived food products from the farm through slaughter. Uniform standards stating the requirements of this program have been developed and federal regulations in support of the program are being developed. This USDA program will serve as a model for the development of other quality and safety initiatives.

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Fermented Liquid Feed: The potential for eliminating enteropathogens from feed

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Summary: The aim of this study was to determine the effect of temperature on the survival of *Salmonella* and *E. coli* in fermented liquid pig feed (FLF). Liquid feed, fermented with *Lactobacillus plantarum*, was challenged with *Salmonella* or *E. coli* (six serovars of each) at 20, 30 or 37 °C. Temperature significantly affected the survival of *Salmonella* and *E. coli*. In FLF containing *ca* 230 mmol L⁻¹ lactic acid the mean decimal reduction time (D) for *Salmonella* was reduced from 157 (±10) min at 20 °C to 12 (±1.5) min at 30 °C and < 5 min at 37 °C. Likewise, the mean D for *E. coli* was significantly reduced from >180 min at 20 °C to 30 (±12.7) min at 30 °C and 21 (±6.2) min at 37 °C. These studies suggest that successful elimination of potential pathogens from liquid feed can be achieved through fermentation with appropriate lactic acid bacteria and temperature control.

Keywords: *Salmonella*, *E. coli*, pigs, lactic acid, *Lactobacillus*

Introduction: Liquid feed (LF) is often fed to grower and finisher pigs in UK and Europe. However, unless steps are taken to prevent it, liquid feed has the potential to be a vector for pathogenic microorganisms. The risk of proliferation of both pathogenic and spoilage organisms in LF can be reduced by fermenting LF with lactic acid bacteria (Brooks et al.,2001). A typical fermented liquid feed (FLF) has a pH of 3.8 – 4 and contains 150 – 250 mmol L⁻¹ lactic acid, which enables it to withstand contamination by other microorganisms including pathogens such as salmonellae. However, extrinsic environmental factors such as temperature may affect both the ability of lactic acid bacteria to grow and produce lactic acid and the survival of enteropathogens in FLF (Beal et al.,2002). The aims of this study were to determine the affect of temperature and fermentation time on the generation of lactic acid and the survival of *Salmonella* and *E.coli* in FLF.

Materials and Methods: A commercial piglet diet was sterilized (25 kGy g irradiation), mixed with sterile distilled water (2.5 water:1 feed), inoculated with *ca* 10⁶ cfu ml⁻¹ *Lactobacillus plantarum* and incubated at 20, 30 or 37 °C for 48, 72 or 96 h. Samples of the resultant FLF's were taken for lactic and acetic acid analysis by high performance liquid chromatography. FLF's were inoculated (in triplicate) with *ca* 10⁷ cfu g⁻¹ of the *Salmonella* serovars: Typhimurium, DT104B(342A), DT104B(342B), DT193(20), Derby(16), Goldcoast(245) and Anatum(41A) and *E.coli* serovars: K88(99), K88(100), K88(101), K99(185), K99(230) and O157:H7. FLF's were maintained at 20, 30 or 37 °C and samples taken at appropriate time intervals for the enumeration of *Salmonella* and *E. coli* using standard plate count techniques.

The decimal reduction time (D) was calculated for each strain.

Results: Different fermentation time/temperature regimes generated different quantities of lactic and acetic acids in FLF. However, there were no statistically significant differences ($p > 0.05$) in lactic acid concentration in feeds fermented at 30 °C for 72 or 96 h and feeds fermented at 37 °C for 48, 72 or 96 h (Table 1). There were significant ($p < 0.05$) differences in D_{30} between strains of *E. coli* and *Salmonella*.

Table 1. Lactic and acetic acid concentration (mmol L^{-1}) and decimal reduction time (D) of *E. coli* and *Salmonella* serovars at 20, 30 and 37 AC in FLF fermented over 48, 72 or 96 h.

| Temperature °C | 20 | | | 30 | | | 37 | | | s.e.d |
|--------------------------|------------------|---------------------|--------------------|--------------------|---------------------|----------------------|--------------------|--------------------|---------------------|-------|
| Ferm Time (h) | 48 | 72 | 96 | 48 | 72 | 96 | 48 | 72 | 96 | |
| Lactic acid | 132 ^a | 192 ^b | 229 ^{bc} | 168 ^a | 224 ^{bc} | 232 ^c | 254 ^c | 251 ^c | 256 ^c | 8.6 |
| Acetic acid | 9 | 11 | 22 ^a | nd | 12 | 22 ^a | nd | 25 ^a | 29 ^a | 3.4 |
| D (min) | | | | | | | | | | |
| <i>E. coli</i> K88(99) | > 180 | > 180 | > 180 | 46.6 ^{a1} | 42.9 ^{a1} | 35.4 ¹ | 25.2 ^{b1} | 23.7 ^{b1} | 22.9 ^{b1} | 1.84 |
| <i>E. coli</i> K88(100) | > 180 | > 180 | > 180 | 60.0 ^a | 56.1 ^{ab} | 50.7 ^b | 26.1 ^{c1} | 23.6 ^{c1} | 17.4 ^{c12} | 1.84 |
| <i>E. coli</i> K88 (101) | > 180 | > 180 | > 180 | 44.2 ¹ | 36.9 ^{a1} | 30.2 ^{a1} | 22.5 ^{b1} | 24.3 ^{b1} | 24.3 ^{b1} | 1.84 |
| <i>E. coli</i> K99 (185) | > 180 | > 180 | > 180 | 35.9 | 21.9 ^{a2} | 19.0 ^{a2} | 22.0 ^{a1} | 16.6 ^{a2} | 15.9 ^{a2} | 1.84 |
| <i>E. coli</i> K99 (230) | > 180 | > 180 | > 180 | 43.7 ¹ | 38.1 ¹ | 28.8 ^{a1} | 22.2 ^{a1} | 14.6 ^{b2} | 14.1 ^{b2} | 1.84 |
| <i>E. coli</i> O157:H7 | > 180 | > 180 | > 180 | 20.6 ^a | 18.7 ^{ab2} | 15.0 ^{abc2} | 12.2 ^{bc} | 9.3 ^{c2} | 10.3 ^{c2} | 1.84 |
| <i>Salmonella</i> :- | | | | | | | | | | |
| DT104B(342A)* | > 1080 | 201.6 | 171.6 | 34.7 | 13.8 ¹ | < 5.0 | < 5.0 | < 5.0 | < 5.0 | 1.49 |
| DT104B(342B)* | > 1080 | 190.8 ¹ | 162.0 ² | 21.7 ¹ | 12.7 ¹ | < 5.0 | < 5.0 | < 5.0 | < 5.0 | 1.84 |
| DT193 (20)* | > 1080 | 238.8 | 147.6 ¹ | 15.4 ^{a2} | 11.6 ^{a1} | < 5.0 | < 5.0 | < 5.0 | < 5.0 | 1.84 |
| Derby (16) | > 1080 | 184.8 ¹² | 150.0 ¹ | 25.8 ¹ | 11.3 ¹ | < 5.0 | < 5.0 | < 5.0 | < 5.0 | 1.84 |
| Goldcoast (245) | > 1080 | 163.2 | 147.6 ¹ | 16.4 ^{a2} | 14.2 ^{a1} | < 5.0 | < 5.0 | < 5.0 | < 5.0 | 1.84 |
| Anatum (41A) | > 1080 | 193.2 ² | 164.4 ² | 15.1 ^{a2} | 10.2 ^{a1} | < 5.0 | < 5.0 | < 5.0 | < 5.0 | 1.84 |

* *Salmonella* Typhimurium serovars < 5.0 = no organisms were detected after 5 min challenge

^{a,b,c} means with the same superscript in the same row are not significantly different ($p < 0.05$)

^{1,2} means with the same superscript in the same column are not significantly different ($p < 0.05$)

Of the *E. coli* strains, O157:H7 appeared to be the most sensitive to the conditions prevailing in FLF. Temperature had a significant ($p < 0.001$) affect on the death rate of both organisms. This was most apparent in FLF's fermented at 20 °C and 30 °C for 96 h; both contained comparable concentrations of lactic and acetic acid (230 and 22 mmol L^{-1} respectively). The 10 °C increase in temperature resulted in a 5–6 fold and > 35 fold increase in the death rate of *E. coli* and *Salmonella* respectively. Increasing the temperature to 37 °C resulted in a further increase in death rate.

Discussion: On most farms temperature of liquid feed is not controlled. However, this study demonstrates that temperature control is important in reducing the risk of enteropathogen transmission via fermented liquid feed. At 20 °C there was little reduction in numbers of *Salmonella* or *E. coli* within the first three hours of inoculation. This is in agreement with previous studies in which FLF was challenged with *Salmonella* species (Beal et al., 2002, van Winsen et al., 2001). In this study, a large inoculum size was used in order to generate D values and it is unlikely that such high levels of contamination would occur on farms. However, even with more realistic numbers of 10 – 100 organisms g^{-1} feed it could take

3 - 18 h to eliminate *Salmonella* or *E. coli* from feed at 20 °C compared with 30 min - 2 h at 30 °C and less than 30 min at 37 °C. Lactic acid concentration also plays an important role the elimination of enteropathogens from FLF and in this study high concentrations of lactic acid were achieved via inoculation with *Lb plantarum*. With proper management, i.e. temperature control and inoculation with lactic acid bacteria to ensure adequate lactic acid production, FLF systems have the potential to play an important role in the reduction of enteropathogens in the food chain.

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Population of a farrowing unit by *Salmonella* negative animals

O 55

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Summary: In order to obtain a better control of *Salmonella* in swine herd, it is important to ensure introduction of negative replacement animals. The objective of this project was to introduce negative sows in a new farrowing unit by use of a protocol based on a combination of bacteriology and serology to select replacement gilts in the finishing unit of origin. Animals were selected from a finishing unit known to be moderately contaminated by *Salmonella* based on previous bacteriological and serological analysis. Based on the results, animals were separated in 2 groups. The first group (group A) consisted of seronegative gilts from *Salmonella* negative pens, designed NP/NS. Group B was composed of seronegative gilts from positive pens, was designed PP/NS and gilts were treated with neomycin to reduce *Salmonella* shedding. All animals were also washed at their arrival in the farrowing unit. Results demonstrated that it is possible to populate new herds by negative animals (NP/NS) coming from a positive herd by selecting animal using bacteriology and serology, and by application of biosecurity and prophylactic measures.

Keywords: biosecurity, prophylactic, serology, excretion, salmonella-free

Introduction: Pork products have been associated with many cases of salmonellosis in human (Beran et al., 1995). Since *Salmonella* is a facultative intracellular pathogen, following the infection, many animals will remain healthy carrier up to the end of the fattening period (Letellier et al., 1999). When stressed (eg transport to slaughter), many healthy carriers will shed the bacteria and contaminate other

animals, trucks and packer's facilities. Carcasses from animals positive to *Salmonella* are three times more likely to be contaminated by this bacteria following the slaughter process (Beran et al., 1995). The control of this foodborne pathogen at the pre-harvest level in swine is thus a critical step to decrease the prevalence of foodborne salmonellosis in humans. In most instances, measures applied on swine farms to reduce prevalence of *Salmonella* have been based on the control of various risk factors and improvement of hygiene. In order to obtain a better control of *Salmonella* in swine herd, it is important to ensure introduction of negative replacement animals. The objective of the study was to develop and verify the effectiveness of a protocol based on a combination of bacteriology and serology to select *Salmonella* negative gilts for population of a new farrowing unit.

Materials and methods: Animals were selected from a finishing unit known to be moderately contaminated by *Salmonella* based on previous bacteriological and serological analysis. Bacteriological status of pens and serological status of animals were evaluated twice, one month and one week before transportation to the new unit, after stress periods such as genetic selection. Bacteriological status of pens was evaluated by conventional enrichment and culture procedures performed using 5 g (5 X 1 g) of feces. Briefly, fecal samples were homogenized in nutrient broth (NB) and incubated 18 h at 37°C. One mL of NB of each specimen in the primary enrichment was transferred to 9 mL of tetrathionate brilliant green and incubated for 24 h at 37°C, for selective enrichment of *Salmonella* spp. Then, one loopful (10 mL) of the selective enrichment media was inoculated in brilliant green sulfa agar (BGS) containing novobiocin at 20 mg/mL and incubated for 24 to 48 h at 37°C. Typical colonies were tested biochemically and tested by agglutination with a polyvalent O-antisera (Poly A1-VI). *Salmonella* isolates were serotyped at the Office International des épizooties (OIA) *Salmonella* Reference Laboratory, Health Canada in Guelph, Ontario. Serological analysis were performed on each gilt using an ELISA test (Maxivet Inc) for detection of antibodies against *Salmonella* (group B, C, N and E). Sows were considered positive at 30% cut-off value (40% value is the positive cut-off for the test and 30 to 39% values are considered doubtful). Based on the results, animals were separated in 2 groups. The first group (group A, n=754) consisted of seronegative gilts (<30% cut-off) from *Salmonella* negative pens, designed NP/NS. Group B was composed of 184 seronegative gilts from positive pens and were designed PP/NS. Based on the antimicrobial agent profile of the *Salmonella* strain recovered from pens, gilts were treated with neomycin (50 mg/kg) during 5 days after transportation. All animals were also washed at their arrival in the farrowing unit and housed separately in individual stall. A sub-population of each group was followed by bacteriological cultures to evaluate the presence of *Salmonella* in selected animals.

Results: In group A, 144 individual fecal samples were collected and all gilts were found negative. In group B, only one sample was positive to *Salmonella*. This gilt was then removed to prevent the dissemination within the room. Strict biosecurity measures were in place during all the experiment.

Table 1. Recovery of *Salmonella* in the farrowing unit after arrival of sows (farm B)

| Group | No of gilts | Percentage of positive animals |
|-----------|-------------|--------------------------------|
| A (NP/SN) | 754 | 0% (0/144) |
| B (PP/SN) | 184 | 1% (1/100) <i>S. Tennessee</i> |

Discussion: So far, most on farm *Salmonella* control programs are targeted against the appropriate management of known risk factors such as presence of rodents, lack in hygiene. However, it is known that the status of incoming animals and the number of sources for replacement animals are important features to consider for appropriate management of *Salmonella* in swine herds (Letellier et al., 1999; Quessy et al., 1999). The identification of sows status is thus critical for the reduction of *Salmonella* in farrowing unit and to produce *Salmonella* free piglets.

Optimal identification of positive animals is thus imperative in order to avoid introduction of positive animal in a herd. Since it may be difficult to obtain replacement animals from herds with a confirmed

negative status for *Salmonella*, we were interested in this study to determine if it was possible to develop a protocol that would allow the population of a negative farrowing unit by use of animals from a herd known to be moderately contaminated by *Salmonella*. Since serology can not detect recently infected animals and since bacteriology can not detect most healthy carriers, we used a combination of serology and bacteriology, ensuring that the last sampling was done after the stress period caused by the selection process.

In the first part of this study, we observed that all sampled animals from NP/NS pens were negative after introduction in the new farrowing unit. Interestingly, it was also possible to populate an almost negative section of the new unit by use of PP/NS animals. To do so, few basic measures were applied to these animals such as a Neomycin treatment in water and the washing of gilts at their arrival to the new unit. In addition, few special precautions such as maintenance of the integrity of pens and biosecurity measures such as changing/washing boots between positive and negative pens, were applied throughout the experiment.

Conclusions: Results obtained in this study demonstrated that it is possible to populate a swine herd with animals negative to *Salmonella* coming from a positive herd by selecting animal using bacteriology and serology combined with application of biosecurity and prophylactic measures. Further studies will be possible on seronegative gilts from positive pens, to investigate their possible resistance to *Salmonella* infection.

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Field trials to evaluate the efficacy of mash feed to reduce *Salmonella* shedding in swine

O 56

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Summary: The objective of this study was to evaluate the effect of corn based mash feed as a pre-harvest intervention strategy to reduce shedding in pigs herds contaminated by *Salmonella* spp. In this study, three nurseries previously found contaminated by *Salmonella* in successive production cycles were selected to evaluate the effect of mash feed. Pelleted feed was administered for the first part of the experiment and mash feed was then introduced in all herds for a period of four production cycles. Samples from pens were cultured to evaluate the prevalence of *Salmonella* in each production cycle. A total of 195 samples for the period corresponding to pelleted feeding and 68 samples for the period corresponding to mash feed were collected. Results indicated a significant reduction of *Salmonella* shedding in herds following mash feed utilization.

Keywords: pelleted, biosecurity, grinding, excretion, incoming animals

Introduction: Among the recognized source and/or risk factors associated with *Salmonella*, presence of rodents, ineffective washing and disinfection protocols, multiple sources of incoming animals and lack in biosecurity are often observed (Quessy et al., 1999). Feed can also be a source of contamination of pig herds. Pelleting of feed was, and is still, considered as a good tool to destroy *Salmonella* when a significant contamination of incomings is known or suspected to be contaminated with *Salmonella*. However, as an adjunct effect, it kills most of the microflora that may help to protect animals if they are exposed to *Salmonella* within the farm environment (Israelsen et al., 1996; Heidenrich and Löwe, 1994). Many studies reported that feed formulation can influence the outcome of the infection in pigs. The objective of this study was to evaluate the efficacy of mash feed as a pre-harvest intervention to reduce shedding in pigs in herds contaminated by *Salmonella* spp.

Materials and methods: Three nurseries, previously found positive to *Salmonella* by bacteriological culture, were followed during successive production cycles in this field study. Corn-based pelleted feed was first given to piglets during 8 production cycles and mash feed (1100 µm) was then introduced in all herds for a period of four production cycles. Pooled 5 g samples of feces from pens (n=8) were analysed in every farm to evaluate the prevalence of *Salmonella* in each production cycle. A total of 195 samples were cultured by conventional enrichment and culture procedures for the pelleted feed period and 68 samples during mash feed administration. Briefly, fecal samples were homogenized in 45 mL nutrient broth (NB) and incubated 18 h at 37°C. One mL of NB of each specimen in the primary enrichment was transferred to 9 mL of tetrathionate brilliant green and incubated for 24 h at 37°C, for selective enrichment of *Salmonella* spp. Then, one loopful (10 mL) of the selective enrichment media was inoculated in brilliant green sulfa agar (BGS) containing novobiocin at 20 mg/mL and incubated for 24 to 48 h at 37°C. Lactose-negative colonies were tested biochemically on urea and triple sugar iron slants. Colonies with reactions characteristic of *Salmonella* spp. were tested by agglutination with a polyvalent O-antisera (Poly A1-VI) and *Salmonella* isolates were serotyped at the Office International des épizooties (OIE) *Salmonella* Reference Laboratory, Health Canada in Guelph, Ontario. No modification was done in the management of herds and sources of incoming animals were not changed during the experiment. No specific therapeutic treatment was administered to these herds during the experiment.

Results: Results indicated that 64% of samples taken during the pelleted feed period were positive for *Salmonella* while 21% of positive samples were found during the mash feed period (table 1). The analysis of data indicated a significant reduction of *Salmonella* shedding in these herds under the mash feed period. A one-tailed Fisher's exact test was used ($\alpha=0.05$).

Table 1. Comparison of *Salmonella* prevalence in pens for pelleted or mash feed period.

| Farm | Pelleted feed period | | Mash feed period | | |
|-------|-------------------------|-----------------------------|-------------------------|-----------------------------|---------|
| | No of collected samples | Percentage of positive pens | No of collected samples | Percentage of positive pens | p value |
| 1 | 68 | 72% | 32 | 32% | <0.001 |
| 2 | 63 | 46% | 24 | 24% | <0.06 |
| 3 | 64 | 73% | 12 | 12% | <0.04 |
| total | 195 | 64% | 68 | 21% | |

Discussion: In the current field situation, even with application strict biosecurity measures and HACCP-based programs, the periodical introduction of healthy carriers positive to *Salmonella* is difficult to avoid. It is thus necessary to take appropriate actions to reduce shedding of contaminated animals when herds are found to be significantly contaminated by this bacterium. Results obtained

in this study indicated that use of mash feed in younger animals may help to significantly reduce the shedding of *Salmonella* in contaminated nurseries. While the exact mechanism associated with this protection is unclear some authors suggested that the microflora of non-pelleted feed act by competitive exclusion. The coarse grinding (1100 μ m) was also found important in the current study to reduce the shedding of *Salmonella*. Use of mash feed can thus be considered as an interesting measure that can be part of a comprehensive plan to control *Salmonella* in swine farms.

Conclusions: Research are currently conducted to assess the efficacy, in field conditions, of mashed feed in older animals and to understand how the size of feed particles can affect the shedding of *Salmonella*.

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Pilot experiment with the aim to reduce salmonella prevalence in pork by logistic slaughter of pigs

O 57

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Summary: A pilot experiment was carried out with the aim to evaluate the possibilities and results of logistic slaughter of pigs (separate slaughter of salmonella-free and salmonella-infected pig herds) under usual circumstances in a Dutch slaughterhouse. During the experiment salmonella-free herds were delivered and slaughtered on Tuesday mornings. Herds delivered on Thursday mornings served as a control group. No significant difference could be found in the number of salmonella-positive

carcasses on Tuesdays and Thursdays. This was mainly caused by contamination of carcasses by contaminated slaughter equipment; 80% of all salmonella strains on carcasses were equal to strains isolated from slaughter equipment. If these salmonella strains were not included in the results, logistic slaughter did result in a significantly lower prevalence of salmonella on carcasses on Tuesdays. It was concluded that separate slaughter of salmonella free pig herds can result in a lower salmonella prevalence on carcasses, but only if slaughter hygiene and especially cleaning and disinfection before and during processing will be improved.

Introduction: In The Netherlands salmonella in pork is responsible for about 25% of all cases of human salmonellosis. Efforts are made to introduce a salmonella control plan in the pork chain. Before introducing such a plan, a pilot experiment was carried out with the aim to evaluate the possibilities and results of logistic slaughter of pigs (separate slaughter of salmonella-free and salmonella-infected pig herds) under usual circumstances in a Dutch slaughterhouse.

Materials and methods: The experiment was carried out in a Dutch pig slaughterhouse, which slaughters 600 to 650 pigs per hour. The experiment consisted of two phases. In the first phase herds with negative or low salmonella prevalence were selected for taking part in the experiment. Selection was based on serological screening of herds for a period of three months (Van der Wolf et al, 2003). In total, 52 herds were selected, which was sufficient to ensure that each week on the same day at least 2000 pigs from the selected herds could be delivered. In the second phase of the experiment the selected herds delivered their pigs during 6 weeks on Tuesday mornings. Pigs from selected herds were delivered to the slaughterhouse before pigs from other herds and both groups were kept separately. Pigs delivered on Thursday mornings served as the control group. The lairage and the slaughterline were cleaned and disinfected thoroughly before pigs were delivered.

To determine the effect of the separate slaughter, the following samples were collected from the slaughtered pigs and the environment: 1200 blood samples, 122 faecal samples from the lorry of each delivered herd, 594 carcass samples, 302 tonsils, 110 swab samples from slaughter equipment before and after slaughter and 60 swab samples from the lairage before pigs were delivered and 60 after the pigs had left the lairage. If the faecal lorry sample of a particular herd was salmonella positive one or more times, then 4 pooled faecal samples were collected on the farm. Blood samples were tested with the Dutch salmonella mix ELISA by the Animal Health Service (see van der Wolf et al, 2003). All other samples were tested for the presence of salmonella according to standard procedures (same as in Swanenburg et al, 2003). Statistics were done with Statistix 7.0.

Results: 39 of the 52 selected herds delivered pigs to the slaughterhouse one or more times during the second phase of the experiment. The number of salmonella-positive carcasses on Tuesdays and Thursdays did not differ significantly. The number of salmonella-positive tonsils on Tuesdays was significantly lower than on Thursdays, indicating that on Tuesdays pigs with lower salmonella prevalence were delivered. 80% of all salmonella strains on carcasses (table 1) were equal to strains isolated from slaughter equipment (*S. bovismorbificans*). If these salmonella strains were not included in the results, logistic slaughter did result in a significantly lower prevalence of salmonella on carcasses on Tuesdays in weeks 4, 5 and 6 of the experiment.

Table 1: *Salmonella* serotypes, isolated from carcasses, phase 2

| Serotype | Percentage of total |
|---------------------------------------|---------------------|
| <i>S. Bovismorbificans</i> | 76 |
| <i>S. Brandenburg</i> | 11 |
| <i>S. Typhimurium</i> (4 phage types) | 9 |
| <i>S. Panama</i> | 2 |
| <i>S. Infantis</i> | 2 |
| Total | 100 |

From the 39 herds that delivered pigs, salmonella was isolated from the lorry faecal samples from 20 herds. In some cases the salmonella serotype that was found in the lorry was equal to the type isolated from carcasses from the same herd. For only 2 of 20 herds the serotypes isolated in the lorries were equal to the salmonella types isolated from farm faecal samples.

Discussion: The results showed that the number of salmonella positive tonsils on Tuesdays was significantly ($p=0,0006$) lower than on Thursdays, which indicates that on Tuesdays less salmonella infected herds were delivered to the slaughterhouse, and the infection pressure was lower than on Thursdays, which was the aim of logistic delivery of herds. However, on Tuesdays the number of salmonella contaminated carcasses was not lower than on Thursdays. There are three possible explanations for this fact. First, not all pigs from the selected herds were salmonella-free, so that cross contamination within and between herds could have happened. Second, the lorries and the lairage were not completely free of salmonella on all days. However, only a few times salmonella types, found in the lorries, were also isolated from carcasses. Salmonella types found in the lairage were hardly isolated from carcasses, so the lorry and lairage were not a major cause for contamination of the carcasses. Thirdly, results showed that cross contamination from slaughter equipment to carcasses took place during slaughter. Salmonella was isolated from slaughter equipment before slaughter started sometimes during the experiment. The salmonella serotype, isolated from the carcass splitter was equal to the serotype that was isolated from 80% of the salmonella-positive carcasses. It seemed that a salmonella "house flora" was present on the splitter. This was shown before in other pig slaughterhouses by Swanenburg et al. (2001) and Dahl (2002). Although the slaughter equipment is cleaned and disinfected daily, some parts of the machines are out of reach of the cleaning, so these parts are never being cleaned, and salmonella can "hide" on these parts.

It was concluded that separate slaughter of salmonella free pig herds can result in a lower salmonella prevalence on carcasses, but only if slaughter hygiene and especially cleaning and disinfection before and during processing will be improved. When new slaughter equipment is designed special attention has to be paid to the fact that it should be possible to easily clean and disinfect the equipment completely during as well as after processing.

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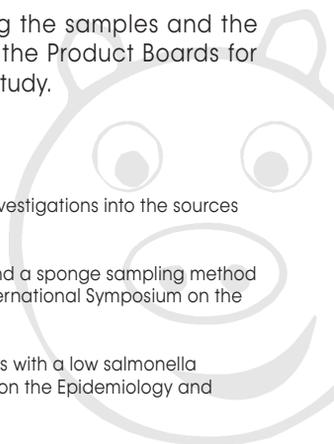
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O 58 Does animal origin and hide status affect microbial contamination in pig carcasses?

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Summary: Contamination in pigs and pig carcasses by *Listeria monocytogenes* and *Salmonella* spp. in an industrial abattoir was co-related with animal origin and their hide status. Animals supplied by farms joining to an integrated quality control program or other pig fatteners were kept in lairage for the night and showered or were slaughtered within few hours. Prevalence in *Salmonella* was similar in animals supplied by farms joining or not the IQC program and *Listeria monocytogenes* was never detected in pigs at bleeding. The differences in the prevalence of *Salmonella*, which varied from 14 to 86% in different stocks of pigs, were related to suppliers and lairage time. The prevalence was much lower (11%) at time of carcass dressing and boning. *Listeria monocytogenes* contamination was very low in pigs at bleeding, but increased after slaughtering (13%) and much more after boning (68%). Any correlation was found between number of Enterobacteriaceae or TMC and prevalence of the pathogens.

Keywords: *Salmonella*, *Listeria*, Enterobacteriaceae, hide status, slaughterhouse.

Introduction: In some abattoirs pigs are showered during the time that they are kept in lairage to make the animals less restless and reducing fighting (Rahkio et al., 1992). The showering prior to slaughter also affect the hide status, animals are cleaner and this may reduce the degree of fouling of the scalding water, but little evidence exist this has an effect of carcass contamination (James et al., 1999). Many pig slaughter enterprises have Integrated Quality Programs, concerning genetic, feeding and farming in order to assure constant meat quality standards for the industry and traceability. Controls on the origin of animals and feed also are important in implementing programs to reduce pathogens contamination at farm level. This study was aimed at determining if the prevalence of *Salmonella* spp. and *Listeria monocytogenes* is different in pigs supplied by pig fatteners joining or not to the IQC program an if it can change, as a consequence of showering/lairage time. Changes in the prevalence of *Salmonella* and *Listeria* were also evaluated during slaughtering and hot-boning.

Materials and Methods: An industrial abattoir located in North of Italy producing more then 1500 pig carcasses per day, which are hot-boned was investigated in this study for four months. Many pig suppliers were joining to an Integrated Quality Control (IQC) which was aimed at assuring origin, genetic, feed quality and farming technology (animals kept indoor in clean well ventilated buildings, supplied with controlled food and water, all-in/all-out) as well as the animal traceability. However, pigs are supplied also by other pig fatteners which collect piglets from many breeders. Pigs arriving in the afternoon were kept in lairage for the night and showered, whereas those arriving in the morning were slaughtered within few hours and were not showered. Therefore four groups of pigs were considered: pigs supplied by farms joining to the IQC program or not, and pigs showered prior to slaughtering or not. For each group twenty pigs were sampled. Five pigs for each stock arriving at the abattoir and four replicates from each group, were sampled at time of bleeding, carcass dressing and boning. Samples were taken by swabbing sterile moistened sponges on the hide of animals (20 cm² in the flank region) during the bleeding time. Two contiguous areas and two sponges were used for each animal. Sponges were moistened with buffered saline added with 0.1% peptone or *Listeria* Enrichment Broth and after sampling they were put in vials containing 100 mL of the same media.

Carcass samples were taken by the excision method reported in the Decision 2001/471/CE. Other samples were taken from shoulders (20 cm² of the skin) at the end of hot-boning. Three samples were taken from each carcass and shoulder. All samples were stored at 0-8 °C. Swabs taken at bleeding were used to detect *Salmonella* and *Listeria monocytogenes*. The three samples taken from carcasses and shoulders were used also for counting *Listeria monocytogenes*, total aerobic bacteria (CMT) and Enterobacteriaceae. The methods reported in the Decision 2001/471/CE were used for CMT and Enterobacteriaceae. *Salmonella* spp., and *Listeria monocytogenes* were detected with official and certified methods of the Regional Veterinary Laboratory (IZS Lombardia ed Emilia). Methods MP 01/001 (modified by ISO 6579:2002) MP 01/002 (modified by ISO 11290-1) and MP 01/003 (modified by ISO 11290-2) were used and results were reported as presence/absence of *Salmonella* or *Listeria monocytogenes* in 20 cm² and *Listeria monocytogenes* colony forming units (CFU) g⁻¹.

A Monte Carlo simulation risk model was developed to define probability distribution of salmonella in animal groups and estimate differences among groups of animals (Fig. 1). A similar model was also used to define probability distribution of *Listeria monocytogenes* and estimate difference in prevalence after processing of carcasses and cutting/boning. Ten thousand iterations were performed for each simulation using @Risk software (Palisade, Newfield, NY) and Microsoft Excel (Microsoft Corp., CA). The prevalence was estimated as a Beta function with parameters 's+1' and 'n-s+1'; Beta (s+1, n-s+1), where 's' is the number of positive samples and 'n' is the total number of animal sampled in each group. Confidence that prevalence in group A is higher than in group B is determined by mean of 10,000 iteration of a cell: '=IF(A>B,1,0)'. The same calculation, which is equivalent to a numerical integration of the different areas described by the prevalence distributions, is used to estimate how big (i.e. > 10%) is the difference in the prevalence: mean of a cell: '=IF(ABS(A-B)>10%,1,0)' (Vose, 2001).

Results: Lower prevalence of *Salmonella* were observed in the groups of pigs that were not kept in lairage for the night and were not showered in comparison with the other groups (Figure 2). The difference was higher than 10% and level of significance was equal to 99%. Pigs supplied by farms joining to the IQC program did not show significant difference with those supplied by other farms (Figure 1). *Salmonella* contamination in pig carcasses and hot boned meat was significantly lower than in pigs at bleeding (Figure 3), whereas, contamination by *Listeria monocytogenes* was higher (Figure 4). Microbial counts used to evaluate the hygiene of slaughtering and cutting operations, namely Total Microbial Counts and Enterobacteriaceae, showed levels below the limits set by the Decision 2001/471/CE. Remarkably, values observed in different stocks of pig carcasses and primal cuts were not indicative of the changes in prevalence of *Salmonella* or *Listeria* observed in stocks after slaughtering and boning (Figure 5 and 6).

Discussion: A study by Morgan et al. (1987) report that salmonella isolations from caecal and carcass surfaces increased with increased lairage time. It is possible that longer pigs stayed in lairage the more likely they can become polluted and infected via cross-contamination. Pen size and hygiene influence the build-up of salmonellas in pigs during lairage (James et al., 1999). Slaughtering operation proved to reduce contamination by salmonella and any significant built-up of *Listeria monocytogenes* was observed at this stage. After cutting and boning operations a relevant building up of *Listeria monocytogenes* contamination, which could be due to environmental contamination (Autio et al., 2000). Counts of mesophilic aerobic bacteria and Enterobacteriaceae, could be indicative of hygienic standards, but are of little value in evaluating different prevalence in *Salmonella* and *Listeria* among pig stocks.

Conclusion: Pigs with low salmonella prevalence should be slaughtered as soon as possible to avoid a build up of salmonella infection. Cold showers could be used to reduce stress, but cleaning is only apparent and risk of salmonella spreading with water during lairage should be avoided. Control of environmental contamination by *Listeria monocytogenes* during hot boning operation should be improved.

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Figure 1

| | A | B | C | D | E | F | G | H | I | | |
|-----------|---|--|---|-----|-------------|-------------|-------------------|---|----|-----------|--|
| 1 | Model to estimate probability distribution for Salmonella prevalence in groups of pigs and difference between groups | | | | | | | | | | |
| 2 | | | s | n-s | p estimate | $P_A > P_B$ | p difference >10% | | mu | results | |
| 3 | Not Showered_IQC | | 13 | 7 | 0.760490082 | | | | | | |
| 4 | Not Showered_NoIQC | | 13 | 7 | 0.556593842 | | | | | | |
| 5 | Showered_IQC | | 15 | 0 | 0.905332076 | | | | | | |
| 6 | Showered_NoIQC | | 12 | 3 | 0.795059055 | | | | | | |
| 7 | showered (P_{A1}) | | 27 | 3 | 0.705265243 | 0 | | 0 | | 0.9912182 | |
| 8 | not showered (P_{B1}) | | 26 | 14 | 0.708952754 | | 0 | 0 | | 0.9226538 | |
| 9 | IQC (P_{A2}) | | 28 | 7 | 0.820519182 | 1 | | 1 | | 0.797713 | |
| 10 | no IQC (P_{B2}) | | 25 | 10 | 0.61691275 | | 1 | 0 | | 0.464828 | |
| 11 | C3:C10 | s=samples that tested Salmonella positive among that tested in the group | | | | | | | | | |
| 12 | D3:D10 | n-s=samples tested in the group-samples that tested Salmonella positive | | | | | | | | | |
| 13 | E3:E10 | p= RiskBeta(s+1;n-s+1) | | | | | | | | | |
| 14 | F7: | IF(P15>P16,1,0) | | | | | | | | | |
| 15 | G8: | IF(ABS(P15-P16)>10%,1,0) | | | | | | | | | |
| 16 | F9: | IF(P16>P17,1,0) | | | | | | | | | |
| 17 | G10: | IF(ABS(P16-P17)>10%,1,0) | | | | | | | | | |
| 18 | H7: | mu=RiskMean(F7) | Confidence that p_{A1} is higher than p_{B1} is determined by mean of cell F7 | | | | | | | | |
| 19 | H8: | mu=RiskMean(G8) | Confidence that difference between p_{A1} is 10% higher then p_{B1} is determined by mean of cell F8 | | | | | | | | |
| 20 | H9: | mu=RiskMean(F9) | Confidence that p_{A2} is higher than p_{B2} is determined by mean of cell F9 | | | | | | | | |
| 21 | H10: | mu=RiskMean(G10) | Confidence that difference between p_{A2} is 10% higher then p_{B2} is determined by mean of cell F10 | | | | | | | | |
| 22 | I7:I10 | mean after 10,000 iterations, LHS sampling | | | | | | | | | |

Figure 2

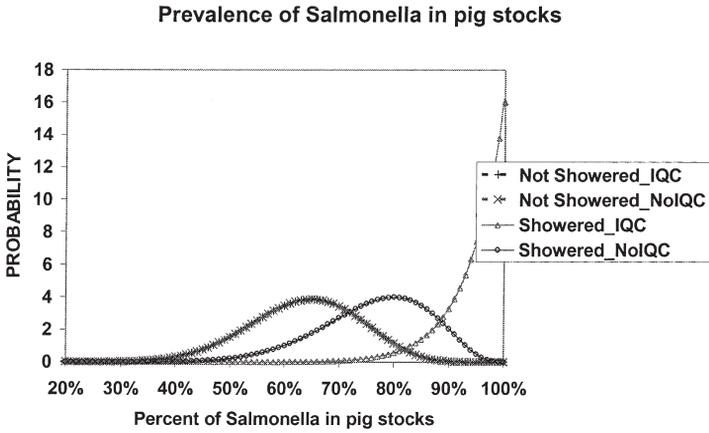


Figure 3

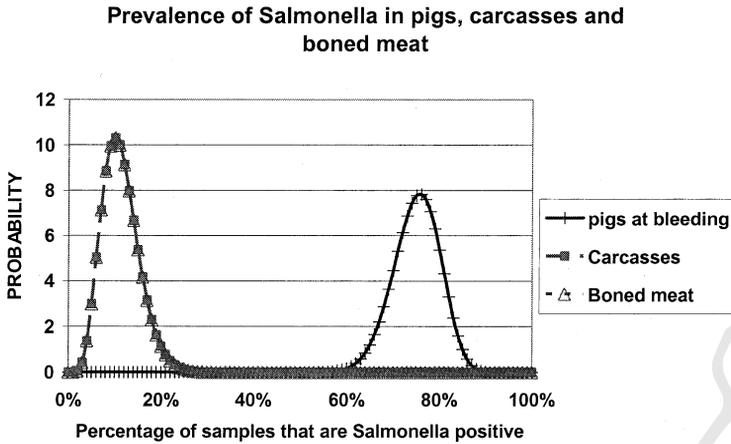


Figure 4

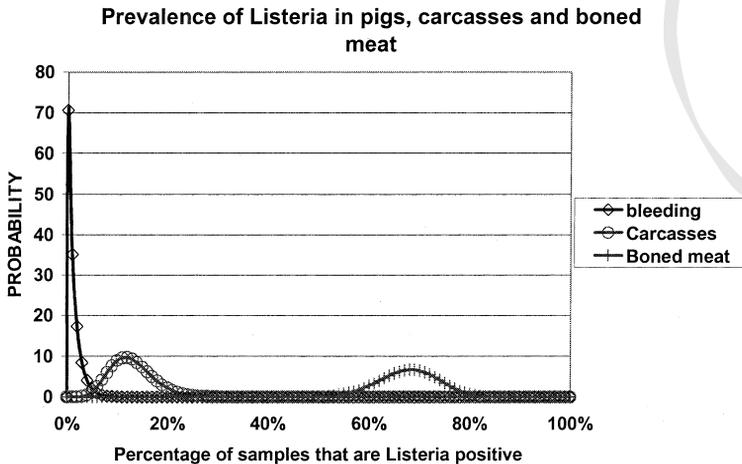


Figure 5 - Changes in prevalence of *Salmonella* spp. during processing

| | A | B | C | D | E | L | M | N | O | P | |
|----|---------------------------------------|---|--|-----|----------------|--------------|-------------------------|----------------------------|-------------------|---|--|
| | | | p | | p difference > | | Enterobacteriaceae mean | | | | |
| | | | s | n-s | estimate | p difference | 10% | Log CFU 20 cm ² | | | |
| 2 | | | | | | | | | | | |
| 3 | All pigs tested | | | | | | | | | | |
| 4 | bleeding (A) | | 53 | 17 | 0.750 | A>B =100% | 100% | | | | |
| 5 | carcass dressing (B) | | 6 | 54 | 0.113 | B>C=49.08% | 7.46% | 1.533 | | | |
| 6 | cutting/boning (C) | | 6 | 54 | 0.113 | | | 1.301 | | | |
| 8 | stocks with various prevalence | | | | | | | | | | |
| 9 | A >>> B = C | | A | 5 | 0 | 0.857 | A>B=99.92% | 99.70% | | | |
| 10 | | | B | 0 | 5 | 0.143 | B>C=49.8% | 49.35% | 1.505 1.744 1.627 | | |
| 11 | | | C | 0 | 5 | 0.143 | | | 1.734 1.472 1.190 | | |
| 12 | | | | | | | | | | | |
| 13 | A < B > C | | A | 0 | 5 | 0.143 | A<B=90.53% | 84.61% | | | |
| 14 | | | B | 2 | 3 | 0.429 | B>C=90.67% | 84.88% | 1.572 | | |
| 15 | | | C | 0 | 5 | 0.143 | | | 1.029 | | |
| 16 | | | | | | | | | | | |
| 17 | A >>> B < C | | A | 5 | 0 | 0.857 | A>B=99.87% | 99.55% | | | |
| 18 | | | B | 0 | 5 | 0.143 | B<C=97.13% | 94.84% | 1.704 | | |
| 19 | | | C | 3 | 2 | 0.571 | | | 0.836 | | |
| 20 | | | | | | | | | | | |
| 21 | A = B < C | | A | 0 | 5 | 0.143 | A>B=50.06% | 49.85% | | | |
| 22 | | | B | 0 | 5 | 0.143 | B>C=50.39% | 50.15% | 1.334 | | |
| 23 | | | C | 0 | 5 | 0.143 | | | 1.055 | | |
| 24 | C4:C23 | | s=samples that tested Salmonella positive among that tested in the group | | | | | | | | |
| 25 | D4:D23 | | n-s=samples tested in the group-samples that tested Salmonella positive | | | | | | | | |
| 26 | E4:E23 | | p= RiskBeta(s+1;n-s+1) | | | | | | | | |

Figure 6 – Changes in prevalence of *Listeria monocytogenes* during processing

| | A | B | C | D | E | J | K | L | M | N | |
|----|---------------------------------------|---|--|-----|----------------|--------------|-------------------------------------|--------|-------------------|---|--|
| | | | p | | p difference > | | TMC mean Log CFU 20 cm ² | | | | |
| | | | s | n-s | estimate | p difference | 10% | | | | |
| 3 | | | | | | | | | | | |
| 4 | All pigs tested | | | | | | | | | | |
| 5 | bleeding (A) | | 0 | 60 | 0.014 | A<B =99.69% | 58% | | | | |
| 6 | carcass dressing (B) | | 7 | 53 | 0.068 | B<C=100% | 100.00% | 3.290 | | | |
| 7 | cutting/boning (C) | | 41 | 19 | 0.672 | | | 3.210 | | | |
| 8 | | | | | | | | | | | |
| 9 | stocks with various prevalence | | | | | | | | | | |
| 10 | A = B < C | | A | 0 | 5 | 0.465 | A<B=50.52% | 50.13% | | | |
| 11 | | | B | 0 | 5 | 0.039 | B<C=90.74% | 84.47% | 3.270 3.46 | | |
| 12 | | | C | 2 | 3 | 0.282 | | | 2.850 3.46 | | |
| 13 | | | | | | | | | | | |
| 14 | A = B << C | | A | 0 | 5 | 0.104 | A<B=50.22% | 51.38% | | | |
| 15 | | | B | 0 | 5 | 0.133 | B<C=99.90% | 99.55% | 3.550 | | |
| 16 | | | C | 5 | 0 | 0.952 | | | 2.970 | | |
| 17 | | | | | | | | | | | |
| 18 | A < B << C | | A | 0 | 5 | 0.359 | A<B=77.01% | 67.69% | | | |
| 19 | | | B | 1 | 4 | 0.206 | B<C=99.37% | 98.38% | 3.070 3.220 3.140 | | |
| 20 | | | C | 5 | 0 | 0.977 | | | 3.330 3.060 3.170 | | |
| 21 | | | | | | | | | | | |
| 22 | C5:C20 | | s=samples that tested Salmonella positive among that tested in the group | | | | | | | | |
| 23 | D5:D20 | | n-s=samples tested in the group-samples that tested Salmonella positive | | | | | | | | |
| 24 | E5:E20 | | p= RiskBeta(s+1;n-s+1) | | | | | | | | |

The intensified control programme for *Salmonella* at Danish swine slaughterhouses

O 59

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Summary: In Denmark, an ongoing surveillance of *Salmonella* in pork has been implemented since 1993. The surveillance has been optimised and extended in order to further reduce the *Salmonella* prevalence in pork. The pork industry has made an agreement with the Danish authorities, that by the end of 2006 the *Salmonella* prevalence in pork must be reduced with 27% compared to the prevalence by the end of 2001. The intensified Control programme for *Salmonella* at Danish slaughterhouses is based upon carcass swabs. The results of the samples are evaluated for each slaughterhouse every month including the results for the last twelve months. Slaughterhouses with a *Salmonella* prevalence of 2.3% or more on individual carcasses are noted, and if a slaughterhouse is noted 4 times during a 6 months period, it is obliged to start an intensified *Salmonella* control programme.

Keywords: pigs, pork, swab samples, *Salmonella* prevalence

Materials and Methods: In Denmark an ongoing surveillance of *Salmonella* in pork has been implemented since 1993. By the 1st of January 2001 the surveillance was revised and is now based upon carcass swabs taken after 12 hours of cooling. From each carcass three areas of 100 cm² are sampled, yielding a total of 300 cm². The areas sampled are 100 cm² on the hind leg near the tail, 100 cm² near the sternum and 100 cm² on the jowl. These sampling areas are the same as the ones described by FSIS, USA, for slaughterhouses, who want to export to the USA. From each slaughterhouse, slaughtering more than 200 pigs daily, 5 carcasses are sampled every day, and the 5 swab samples are analysed as one pooled sample. The results are evaluated for the latest 11 days of slaughter, and if more than one sample is positive actions must be taken. This surveillance will reveal acute *Salmonella* problems.

By the 1st of May 2002 an intensified control programme for *Salmonella* at the slaughterhouses was introduced. The aim of this programme is to identify slaughterhouses that have an increased prevalence of *Salmonella* over a period of time. The intensified control programme is based upon the same samples as described above, but here the results are evaluated monthly including results for the latest 12 months. This period will provide a sufficient number of samples to ensure statistical confidence, when a slaughterhouse is noted. Slaughterhouses with a *Salmonella* prevalence of 2.3% or more on individual carcasses are noted and if a slaughterhouse is noted 4 times during a 6 months period it is obliged to start an intensified control programme.

When an intensified control programme is started, the slaughterhouse has one month to take samples to:

- Identify the cause for the *Salmonella* contamination if possible
- Work out an intervention plan
- Implement the necessary initiatives

Within the following 6 months the slaughterhouse must document a lasting effect of the initiatives taken. This can be done in two ways: either the *Salmonella* prevalence is again below 2.3% or the slaughterhouse has none or only one positive sample monthly in four months out of the 6 months. If the time limit is not kept the authorities can demand further initiatives taken.

Discussion: The pork industry has made an agreement with the Danish authorities, that by the end of 2006 the *Salmonella* prevalence in pork must be reduced with 27% compared to the prevalence by the end of 2001.

For the Danish Bacon and Meat Council the first year of experience with the intensified controlled programme has shown, that as soon as a slaughterhouse is noted for the first time, a major programme is started at the slaughterhouse in order to locate the source of *Salmonella* contamination. With a sporadic prevalence with 2 or 3 positive samples a month, this work can be very difficult and requires many samples taken over a period of time. This sampling often includes samples from both the unclean and the clean part of the slaughter line.

An increased prevalence of *Salmonella* may also be caused by introduction of automatic equipment or by education of new staff on the slaughter line, but these circumstances seldom influence the slaughter hygiene for more than one or two months.

The initiatives taken to reduce the *Salmonella* prevalence are highly individual and vary from slaughterhouse to slaughterhouse. Some are improvements or changes in routines that can be implemented immediately. Other initiatives are long term investments, that takes time to implement.

During the first 14 months after implementation five slaughterhouses out of 19 slaughterhouses members of the Danish Bacon and Meat Council had to start an intensified control programme. Four of these slaughterhouses are out of the intensified programme again and one is still working on it within the time limit. So far, all the slaughterhouses have been able to reduce their *Salmonella* prevalence on the carcasses within the time limit.

The *Salmonella* prevalence in pork from slaughterhouses members of The Danish Bacon and Meat Council was 1.7% by the end of 2001. By the end of 2002 the prevalence was reduced to 1.5%. Furthermore the number of Danes with salmonellosis caused by pork was reduced from 166 cases in 2001 to 78 cases in 2002 (Anonymous, 2003).

References:

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O 60 EXPOSURE ASSESSMENT OF FOODBORNE PATHOGENS IN PORK IN BELGIUM

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Summary: The aim of this study was to assess the exposure of the most incident foodborne pathogens in the Belgian meat production chain. The prevalence of *Salmonella*, *Campylobacter* and *Listeria monocytogenes* were evaluated in carcasses (swabs), retail cuts, minced meat and meat products of pork. The investigation was made each year since 1997, using official methods from the Ministry of Public Health for *Salmonella* and *Campylobacter* and the Vidas *Listeria monocytogenes* method. More than 10 % of each matrix were contaminated with *Salmonella*. For minced meat and meat products, the contamination rate were respectively round 20 % and 3 - 6 % for *Listeria monocytogenes*. Under 5 % of minced meat samples were positive for *Campylobacter*. For minced meat, the contamination has also been assessed according to the location of sampling (agreed, low capacity establishments or retail level). The characterisation of bacterial species allows the comparison between meat and human isolates.

Keywords: *Salmonella*, *Campylobacter*, *Listeria monocytogenes*, meat, surveillance

Introduction: *Campylobacter* and *Salmonella* are the most common causes of bacterial gastroenteritis in terms of reported incidence by the public health services. *Listeria monocytogenes* is regularly implicated in severe foodborne infections. The assessment of their exposure is essential for an efficient risk assessment program.

Materials and methods: Since 1997, the Belgian zoonoses surveillance program has assessed the national contamination with *Campylobacter*, *Salmonella* of carcasses (600cm²), retail cuts (25g), minced meat (25g) of pork, and meat products (ham and pâté, 25g). The prevalence of *Listeria monocytogenes* is evaluated in pork minced meat since 2000.

Round 300 samples were taken for each matrix.

The detection of *Campylobacter* and *Salmonella* have been carried out with official methods from the Ministry of Public Health (SP-VGM003&4), using Preston broth and mCCDA for *Campylobacter* and BPW and Diassalm for *Salmonella*. The detection of *Listeria monocytogenes* has been carried out with the AFNOR BIO-12/3-03/96 Vidas *Listeria monocytogenes* method followed by a chromogenic medium.

Results: More than 10 % of samples from each matrix were contaminated with *Salmonella*. For minced meat, the contamination rate was round 20 % for *Listeria monocytogenes* and below 5 % for *Campylobacter*. Between 3 and 6 % of ham and pâté were contaminated with *Listeria monocytogenes*. The prevalence and evolution since 2000 are given in Figures 1 and 2.

In 2002, minced meat is more contaminated with *Salmonella* at the retail level than in the producing establishments, unlike *Listeria monocytogenes* which is more present in low capacity establishments.

Discussion and conclusion: *Salmonella* and *Campylobacter* are frequently isolated in pork carcasses and cutting meat. The isolated strains belong to the same serotypes than these isolated from animals and human.

The contamination rate is lower in minced meat, but the contamination with *Listeria monocytogenes* shows that appropriate application of hygiene is a basic requirement for its control.

Acknowledgements: The Belgian Federal Agency for the Safety of the Food Chain (Ministry of Public Health) financially supported this study.

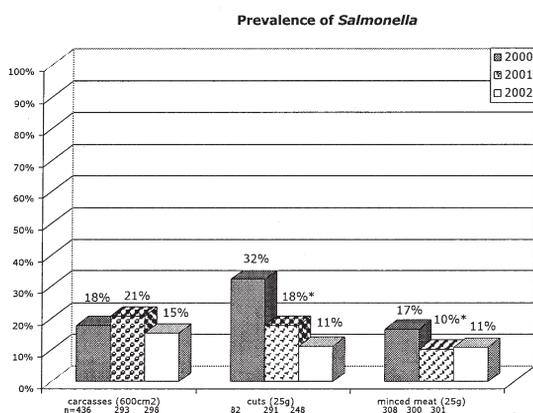


Figure 1: Prevalence of *Salmonella* in pork meat between 2000 and 2002. The "*" shows that the difference between 2000 and 2001 ("*" on 2001) or between 2001 and 2002 ("*" on 2002) is significant ($p < 0,05$). Without any mention, the difference is not significant.

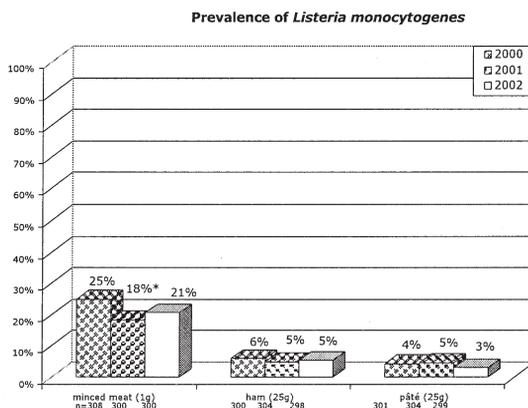


Figure 2: Prevalence of *Listeria monocytogenes* in pork meat between 2000 and 2002. The "*" shows that the difference between 2000 and 2001 ("*" on 2001) or between 2001 and 2002 ("*" on 2002) is significant ($p < 0,05$). Without any mention, the difference is not significant.

O 61 IMPROVING THE MEAT INSPECTION BY AN INTEGRATED QUALITY CONTROL SYSTEM

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Summary: Measures concerning the production of microbiologically safe meat can be divided into those guided by the more or less classical, rigid "legislative" approach and by a more flexible "scientific" approach based on risk analysis.

Therefore intervention should not unduly focus solely on the abattoir or food processing stages as is done with the classical, rigid "legislative" system, but should also target the risks associated with preharvest production stages. A comparison between the "legislative" and the "scientific" approach shows that properly structured HACCP-like systems, applied from farm to fork, as proposed by the new EU legislation and the Dutch implication of an integrated quality control system offer the best available approach to food safety assurance.

Keywords: HACCP; Databank; Salmonella; sampling; pork; decontamination

Introduction: The infection and contamination of pork and pork products by pathogenic bacteria have often been epidemiologically linked to food borne illness in humans. Risk analysis shows that microbial organisms on pork represent the greatest risk to public health. The level of exposure of consumers to microbiological hazards in fresh pork is unlikely to be reduced significantly by the detection and removal of gross abnormalities in the tissues examined, as is done by the to-day's meat inspection. Among the agents involved in pork are *Salmonella* spp., *Campylobacter* spp., *Yersinia enterocolitica* and *Listeria monocytogenes*. Most of the research on pathogens in pork is focussed on *Salmonella*. Inspection at the end of the production-line is not designed or equipped to detect symptomless carriers of zoonotic agents or residues and may be in some cases even contra-productive regarding the hygienic aspects of the production process (Anon. 2000). In modern

animal husbandry large numbers of pigs are raised under optimised hygienic conditions. These conditions however do not guarantee pathogen-free pigs at slaughter. Other live animals and the environment during transport and the period prior to slaughter may serve as a source of pathogenic micro-organisms, which in turn contaminate carcasses during the slaughtering process as well as meat products during further processing, storage and handling.

Integrated Quality Control System: In an integrated quality control system information from the farm is an essential element to ensure safe meat (Anon. 2000). Traceability for all animals is a prerequisite. The competent veterinary authority should collect data using harmonised sampling methods. The information stored in a central databank should cover:

- the nature and origin of the animal feeding,
- the health status of the animals at the farm,
- the use of veterinary medical products,
- the results of any analysis carried out on samples taken at the farm as well as at the slaughterhouse,
- results of slaughterhouse data regarding ante- and post-mortem findings

This information is needed by the meat inspection as well as by the slaughterhouse management as a tool for steering the HACCP like system. In such an integrated system it is possible to allow a visual post-mortem system only. The advantages of omitting particular measures such as palpation and slicing, mentioned in the to-day meat inspection and replace them by only visual inspection are: reduction of cross contamination; reduction of unnecessary damage to the carcasses; better application of resources to more appropriate sanitary measures.

Animals lacking the above-mentioned information cannot be accepted in such control system but have to be slaughtered separately. In addition they will undergo extensive post-mortem inspection and sampling for further laboratory examinations. The farmer has to pay for the cost of the extra analyses and inspection labour.

Presence of Salmonella: The prevalence of *Salmonella* differs between slaughterhouses and sampling days and is also dependant upon which part of the pig is sampled. This was illustrated by a survey conducted by Swanenburg et al. (2001) of 925 slaughter pigs sampled at six different locations. The highest prevalence of *Salmonella* was observed in the rectal contents (25.6%), whereas the lowest prevalence of *Salmonella* was observed on the carcasses (1.4%). The prevalence of *Salmonella* was 19.6 % in tonsils, 9.3 % on livers, 9.3% on tongues and 9.3 % in mesenteric lymph nodes. *Salmonella* was isolated from one or more samples of 47 % of the pigs. *S. typhimurium* was the most frequently isolated serotype. Based upon these observations, the results of *Salmonella* isolations of slaughtered pigs should always be carefully interpreted, with due account being given to which kind of sample has been examined.

Control in slaughter lines: Regarding microbiological Food Safety Requirements (FSRs) a distinction can be made between hygiene guidelines and guidelines to prevent pathogenic micro-organisms arising in foods of animal origin.

Regarding the hygiene guidelines, an EU Commission Decision of 8 June 2001 has been published to introduce compulsory testing and evaluation of the total viable counts and Enterobacteriaceae on carcasses and working surfaces (Anon.,2001). The daily log mean value for acceptable results for samples taken by the destructive method for pigs must be less than 4.0 log N total viable counts per cm² and for Enterobacteriaceae less than 2.5 log N per cm² (Table1). Between 5 and 10 carcasses should be sampled on a single day. Samples should be pooled from ham, back, belly and jowl of the tested pig carcass. Swab sampling removes only a proportion of the total flora present on the meat surface (Snijders et al, 1984). Where methods other than the destructive method are used, the microbiological performance criteria must be established individually for each method applied in order to relate them to the destructive method.

Table 1: Daily log mean value for marginal and unacceptable results for bacterial performance criteria for pig carcasses (cfu cm²) for samples taken by the destructive method

| | Acceptable range | Marginal range (> m but <M) | Unacceptable range (>M) |
|--------------------|------------------|--------------------------------|----------------------------|
| Total viable count | <4.0 log | 4.0 log –5.0 log | >5.0 log |
| Enterobacteriaceae | <2.0 log | 2.0 log- 2.5 log | >2.5 log |

The method used for the bacteriological sampling for checks of cleaning and disinfection efficiency in pig slaughterhouses and cutting plants has also been described (Table 2). The use of the contact plate method and the swab technique is limited to the testing of surfaces, which are cleaned and disinfected, and are dry, flat, sufficiently large and smooth.

Table 2: Mean values for the number of colonies for testing of surfaces

| | Acceptable range | Unacceptable |
|---------------------|--------------------------|-----------------------|
| Total viable counts | 0 – 10 / cm ² | >10 / cm ² |
| Enterobacteriaceae | 0-1 / cm ² | >1 / cm ² |

While these criteria facilitate the control of general hygiene in meat plants, they do not address the question as to whether or not a carcass may be deemed to be free of pathogenic bacteria of human importance. There is therefore a need for the development of guidelines for pathogenic micro-organisms not alone on carcasses but also in food animals at various stages in the course of production. Such guidelines should be based on reliable data and take account of the prevalence of these hazards. This is clearly illustrated by Swanenburg et al 2003.

Origin of contamination: Experiments carried out in Dutch pig slaughterhouses which slaughter 400 to 650 pigs per hour showed that pigs originating from a *Salmonella*-infected farm have a higher chance to end up as the primary source of *Salmonella*-contaminated pork and are also a substantial source of the contamination for the environment, trucks, lairage, slaughter-line and pigs from other herds. A major factor which leads to the contamination of pork with *Salmonella* is the exposure of pigs to infection in the lairage in slaughterhouses. Herd serology is significantly associated with the occurrence of *Salmonella* in rectal contents and lymph nodes of the pigs. This parameter can be used to distinguish between the *Salmonella* risks posed by individual herds and farms. Sero-negative herds should not be slaughtered together with sero-positive herds in the same slaughterhouse. Inter-mixing of animals as well as meat derived from pigs from sero-positive and sero-negative herds somewhere in the production and processing chain will not result in a sufficient solution of *Salmonella* contamination of the final product.

There is now a good understanding of the possibilities for addressing the *Salmonella* problem in pork production. However, contamination of pigs with *Salmonella* should be avoided in all phases of the pork production chain (Lo Fo Wong and Hald,2000).

The Danish *Salmonella* Surveillance and Control Programme, which has been in use since 1995, is based on serological testing of the *Salmonella* status of almost every pig farm. This programme has resulted in a reduced number of highly infected farms, but so far has not resulted in a substantial decrease in the rate of *Salmonella* contamination of pork. Control measures such as the avoidance of direct and indirect contact between *Salmonella*- free and *Salmonella*-positive herds are not yet implemented in this programme. First and foremost, such contact either direct or indirect, between different herds must be prevented along the entire pork production chain, and especially between known *Salmonella*-free and *Salmonella*-infected herds, so that no cross contamination between

herds can occur. Likewise, salmonella-free animals must not be exposed to *Salmonella*-contaminated environments such as trucks and lairages of slaughter houses where such animals may become infected by taking up contaminated water or faecal material from ramps, walls and floors. It is essential that control measures involving the farm as well as the transport, lairage, slaughter and deboning phases, be effectively introduced.

What has been neglected?

Since it is acknowledged that the dressed carcass harbours a complex microflora on its surface, additional on-line microbial decontamination procedures may be required. Such procedures are currently under consideration by both the meat industry and the EU authorities. However, their implementation on slaughter-lines in the EU has not met approval to-date. Yet the only realistic approach to de-contamination of a dressed carcass is the treatment of carcasses at the slaughter-line using methods as heat, chemical treatment or ionising irradiation. Of these, chemical treatment using approved substances (e.g. organic acids and trisodium phosphate) which have met all the necessary safety requirements, appears to be the preferred option. Such an approach has already received the approval of the USDA.

Although the food industry including farmers have the responsibility for the production of safe meat, failures in the supply chain will occur from time to time. Finally, it is very important that the information and advice given in consumer education programmes and on food labels regarding pathogenic micro-organisms is accurate and presented in a user-friendly format, so as to ensure that consumer can play his or her role in preventing the transmission of foodborne hazards.

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O 62 Prevalence of *L.monocytogenes* and *Listeria* spp., in the environment and raw meat products during pig slaughtering, deboning and meat cutting operations.

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Summary: From 9/2001 –6/2002 we estimated the prevalence of *Listeria monocytogenes* and *Listeria* spp., in the environment, and raw pork products of a meat plant. Of 41 environmental samples taken before and after initiation of slaughtering, in 2 visits, 10.7% and 7.7% of the samples respectively harbored *L. monocytogenes*. In each of 2 additional visits we collected 45 samples each time from carcass surfaces. *L. monocytogenes* was present 2.2% and 2.2% of the samples respectively. Of 109 environmental surface samples from the deboning room before and 104 taken 2-3 h after the beginning of the operation 3.7% and 5.8% harbored *L. monocytogenes*. Of 132 environmental surfaces samples taken before and 125 after the initiation of work in a special working area handling the cutting and packaging of modified atmosphere (MAP) consumer size meat cuts 3% and 5.6% harbored *L. monocytogenes*. Of 35 wholesale meat cuts from imported meat collected in the central deboning and cutting room 34.3% harbored *L. monocytogenes*. Finally of 201 consumer size MAP products prepared from the company's own pig carcasses, 6% harbored *L. monocytogenes*. The results indicated the low prevalence of *L.monocytogenes* on local origin carcasses and MAP cuts prepared from such carcasses under strict sanitary conditions. Cross contaminations of equipment and worker's hands from imported meats may result in excessive contamination of meat cuts whether the meat is imported or local.

Keywords: *Listeria monocytogenes*, prevalence, pork, sanitation, *Listeria* spp.

Introduction: Listeriosis mostly of sporadic nature remains a rare but serious human health problem. Meatborne outbreaks, some due to pork and processed pork products have been reported recently. Due to the psychotropic nature of the *L. monocytogenes* and its generally greater resistance to environmental and processing parameters, as compared to other foodborne non-sporoforming pathogens, this microorganism has created serious concerns for the food industry and regulatory agencies. Once processing plants are contaminated certain strains become established and their elimination is difficult (Tompkin, 2002). The prevalence of *L.monocytogenes* in raw meats including pork can be as high as 50-100%. It is frequently isolated also from minimally processed refrigerated foods (Ryser and Marth, 1999). In this study we estimated the prevalence of *L.monocytogenes* in a plant of a vertically integrated company supplying the pigs as well as importing fresh pork for further processing. The study became a necessity because the company introduced MAP to extend the shelf-life of special pork cuts and other raw products originated only from the company's pigs.

Materials and Methods: ISO 11290:98:1 methodology for recovery of *L. monocytogenes* and *Listeria* spp for meat products was used. For environmental testing sponges (TECRA environmental swabs) were used to swab 20X20 cm² areas. The sponges in their original container were transported for incubation in the laboratory where 20 ml of 1/2 Fraser Broth were added to each and incubated for 24 h at 30°C. The following steps were similar to meat handling. Carcass surfaces were sampled using a sterile 20X20 cm individually wrapped cotton pad to swab the neck, the back and the thigh area of each carcass. The swabs were placed in a plastic Stomacher bag containing 200 ml Buffered *Listeria* Enrichment broth with 0.1% Tween 80. In the laboratory they were handled as the other samples.

Results and Discussion: From 9/2001 –6/2002 we visited the plant 7 times. Of 28 environmental samples taken before and 13 after initiation of slaughtering in 2 visits *L. monocytogenes* was present in 10.7% and 7.7% of the samples respectively. Overall *L. monocytogenes* prevalence was 15.8% and 4.5% during the 1st and 2nd visits respectively. In each of two other visits we collected 45 carcass surface samples after the processing of 75-100 pigs originating from the company's nearby farm. The carcasses were sampled before and after the scalding tank, after dehairing and before and after final wash. The overall prevalence during the 1st visit for *L. monocytogenes* and *Listeria* spp was 2.2 and 4.4% respectively. The corresponding prevalence during the 2nd visit that followed extensive improvements in sanitation was 2.2 and 0%. No *L. monocytogenes* was found on carcasses after the final wash. In 6 additional visits to the deboning room we collected 109 environmental samples before and 104 samples 2-3 h after initiation of operations. In this room carcasses of the company's own production and imported meats were handled at the beginning of the study. The prevalence of *L. monocytogenes* on the surfaces of saws, teflon, cutting boards, floors, plastic crates, knife sharpeners, knives and worker's hands before the initiation of the operation was 9.1%, 0%, 16.7%, 12.5%, 0%, 0% and 0% respectively. The corresponding prevalence during operation was 0%, 0%, 12.5%, 0%, 14.3%, 0%, and 14.8%. The overall *L. monocytogenes* and *Listeria* spp prevalence was 4.7% and 33.3% respectively. The prevalence of *L. monocytogenes* for the 1st to the 6th visit before initiation of operations was 42.9, 8.3, 0, 0, 0 and 0 % respectively. The corresponding figures for samples taken during operations were 33.3, 7.7, 0, 0, 4.4 and 0%. Extensive improvements in sanitation were initiated after the 2nd visit. The room handling the consumer size MAP meat, a major raw meat product of the company was sampled 7 times. We collected 132 and 125 environmental samples before and after initiation of operations respectively. The prevalence of *L. monocytogenes* on the surfaces of teflon cutting boards, floors, meat cutting machine knives, worker's knives and worker's hands before and after the beginning of processing was 2.6%, 9.1%, 4.3%, 0%, 0%, and 0%, 12.0%, 16.7%, 0% and 3.6% respectively. The overall *L. monocytogenes* and *Listeria* spp prevalence before and after the operations started was 3, 4.3% and 5 and 16.9% respectively. The prevalence of *L. monocytogenes* and *Listeria* spp in 35 wholesale meat cuts from imported meat, deboned in the central cutting room was 34.3% and 40% respectively. The corresponding prevalence in 201 consumer size MAP products originating from the company's carcasses handle in MAP room was 6% and 22%. Identified factors contributing to environmental and product contamination included: 1) New employees, unfamiliar with the operation and *L. monocytogenes* controls, moving from other departments to the deboning and processing locations or to cleaning and sanitizing equipment; 2) Personnel handling processed products after working in contaminated areas without following instructions for sanitary handling of products; 3) Periods of heavy production making sanitation practices difficult; 4) Meat products delayed in processing lines thus supporting microbial growth; 5) Imported meats with high *L. monocytogenes* prevalence processed at the beginning of a working day thus cross contaminating equipment and eventually low *L. monocytogenes* prevalence local meats; 6) Personnel traffic patterns not following a defined schedule resulting in cross contaminations from dirty to clean areas and products; 7) Cleaning equipment parts on the floor; 8) Starting processing operations before the working environment was fully dry after the cleaning and sanitizing operations; 9) Waste bins, in the cutting and packaging area not maintained cleaned. Overall the study demonstrated the low prevalence of *L. monocytogenes* on the carcasses and MAP meats originating from local animals and processed under strict sanitary conditions. Improvements in sanitation and initiation of strict preventing measures decreased significantly the prevalence of *L. monocytogenes* as indicated by the results of the later visits as compared to the early visits.

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Pork safety and quality through livestock welfare: 1. Welfare of pigs on the farm

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Summary: In this paper the impact of animal welfare on commercial pig farms to pork safety and quality is widely discussed and analyzed. The variety of stressors associated with farm practices, such as animal health and nutrition, breeding and reproduction, housing and environment, their interactions, the inputs and outputs, is presented for both the determination of on-farm welfare indicators and the estimation of their effects on pork safety and quality. Risk analysis and HACCP principles are applied as a valuable adjunct to farm practice in order to organize the information and analyze the representation of physical processes in quantitative mathematical terms, so that welfare indicators can be evaluated and predictions can be made. Finally, this study concludes by supporting the decision making process at farm level oriented towards animal welfare and food safety and quality, with supporting farm experience and scientific literature.

Keywords: HACCP; indicators; assurance scheme; animal handling; food policy

Introduction: Welfare was defined by FAWC in the U.K. taking into consideration five freedoms: 1. Physiological freedom (absence of hunger and thirst) 2. Environmental freedom (adapted facilities) 3. Sanitary freedom (absence of diseases and fractures) 4. Behavioural freedom (the possibility to express normal behaviours) 5. Psychological freedom (absence of fear and anxiety). While this definition may be applied to a relatively long period of rearing (6 month for finishing pigs and 3 to 4 years for sows), enabling the animals to learn, it can hardly be applied before slaughter (e.g., fasting period, transport), especially in the last 24 h of the life of a pig, when the changes in the environment are many and fast. In these conditions, welfare of the pig may be defined as its state of stress (fear, anxiety) due to the new environmental factors to which is submitted. The safety and quality of animal products are strongly dependent on animal welfare (Pont and Maner, 1984). Animal welfare-related factors that may compromise the safety of raw products include microorganism contamination and product content in antibiotics, pesticide and other chemical residues, environmental pollutants (heavy metals), etc. In the fresh meat production poor welfare results both in loss of yield and sales through pork quality defects (P.S.E. and D.F.D meat). Despite the fact that field research in animal welfare on the farm has received considerable attention in recent years and considerable progress has been made, important and fundamental questions concerning the relationships between animal welfare and food safety and quality still remain open. Moreover, there is a lack of on-farm assurance indicators enabling the integration of behavioural, physiological and productivity parameters, so that their effects on the safety and quality of animal products could be estimated (Sossidou, 2002).

The purpose of this study is to identify the on-farm hazards, which potentially result in poorer safety and quality meat.

Materials and Methods: Hazard Analysis and Risk Analysis are used to identify the on-farm hazards (Mortimore and Wallace, 1994, Jensen and Unnevehr, 1999, OIE, 2002). The data were derived from the preliminary surveys of an Integrated Project submitted to the EC 6th Framework Programme for the Research and Technological Development in the thematic priority of Food Quality and Safety (FP6-2002-FOOD-1-Proposal No 506414), relevant literature and other sources, including expert opinions, personal interviews and farm experiences gained over the last year during a post-doctoral thesis

on Farm-Animal Welfare in Greece founded by the State Scholarships Foundation.

Results and Discussion:

I. Pork safety and quality can only be assured on farm by a holistic farm approach to managing animal well-being. This approach requires multidisciplinary inputs in order to be successful since it involves many aspects of veterinary and animal production science. The development of practical and reliable welfare measures for health and nutrition, breeding and reproduction, animal husbandry, housing and environment on pig farm are therefore required.

II. From our preliminary studies, it became evident that a strong link between husbandry factors exists, such as stocking rate, environmental stressors, sanitation, and meat safety although these factors are rather interactive than additive. Factors which have been demonstrated to be influential in improving health and performance and reducing risks include selection of high health stock, maintenance of health barriers, vaccination strategy, multi-site production, depopulation and cleaning of buildings, all-in all-out procedures within housing systems, appropriate cleaning and disinfection between batches to reduce carryover of infection, diet quality, air quality, thermal environment, airflow pattern, reduction in stress from overstocking, relocation, poor handling and social regrouping.

III. Like other areas of policy, assessment of farm animal welfare and food assurance policy must include considerations of legal, ethical, economic and technical aspects (Bennett, 1996, Sandoe and Simonsen, 1992). Science may be able to determine what constitutes better or worse welfare for animals from a biological point of view, but it is a collective decision of society that determines what is considered to be "good" or "bad" animal welfare from an ethical perspective, i.e. what is considered to be acceptable or unacceptable in relation to livestock production practices and handling of animals. On the other hand, concerns about farm animal welfare may also vary considerably throughout the European Union, as related to ethics, socio-economics, legislation, training and information. Statistical data on these differences are therefore of enormous importance.

IV. Pig enterprises pose additional problems because of their production cycle that tends to be more integrated and long term, making isolation of individual cohorts of animals less easier in practice. To introduce animal welfare considerations into the pig industry, it is necessary to face these challenges, in order to resolve uncertainties concerning the exact nature of pig welfare requirements and identify the ways these requirements can be fulfilled under commercial conditions as well as to provide scientific data to help ensuring that decisions are not taken on the basis of subjective or emotional considerations (Sossidou et al., 2003). Concepts for systematic product safety control have been developed and applied in the food industry [HACCP, SSM (Supportive Safety Measures)]. Such approaches can be readily modified for application on farm (Defra, 2003).

IV. Regarding the indicators already drawn up, a number of organizations (Cost Report, 2003), both national and international, have tried to produce an index depicting the fulfillment (or not) of the animal welfare issues on the farm and therefore the production of safe and high quality products. Anyway, across Europe, the objectives for Food Safety and Quality have already been adopted to the Regulation 178/2002/EC.

Conclusions :

- A holistic farm approach approach is required for the identification and the assessment of pig welfare on the farm and its effects on the safety and quality of pork meat.
- It is important to include considerations of legal, ethical and economic aspects in animal welfare research.
- The HACCP concept should be readily modified for on-farm implementation.
- The development of objective data is required for the assessment of on-farm welfare

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O 64

EFFECT OF PRE-SLAUGHTER HANDLING AND SEROLOGY ON *SALMONELLA* IN PIGS

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Summary: This study investigated the combined effect of herd sero-prevalence, time-off-feed prior to slaughter and transport distance to the abattoir on *Salmonella* spp. infection in slaughter pigs under Australian marketing scenarios. Ten herds situated either < 125 km or > 500 km from the abattoirs were monitored at slaughter over a 12-month period both serologically (Australian *Salmonella* spp. mix-ELISA) and by caecal culture. On 4 occasions (seasonally) each farm withdrew feed from three groups of slaughter pigs so they were off feed for 12-18 hours, 18-24 hours and >24 hours prior to slaughter, including transport and lairage times. For herds <125 km from the abattoirs, *Salmonella* spp. infection decreased significantly with an increase in the period between last feed and slaughter. For herds > 500 km from the abattoirs, *Salmonella* spp. infection increased significantly with an increase in the period between last feed and slaughter, other factors apparently overriding the protective effect of fasting. Herd sero-prevalence was not a significant risk factor for caecal positivity.

Keywords: Australia, time-off-feed, transport distance, sero-prevalence, caecal

Introduction: This study investigated the ecology of the major foodborne hazard, *Salmonella* spp. during the period immediately leading up to slaughter. The association between herd sero-prevalence and culture positivity at slaughter has already been established (Dahl and S. erensen, 2001). Rather than duplicating that work, this study aimed to evaluate the relative contribution

of herd serological status and local marketing procedures (transport distance/time-off-feed). This data would then allow comment on the value of herd sero-monitoring to the Australian pig industry and the importance of other factors.

Methods: Initial serological screening was conducted on 24 South Australian herds, selected on the basis of their distance from the abattoirs and their ability to send a minimum of 60 finisher pigs directly to slaughter at one time. Ten of these were selected and their sero-prevalences monitored over a 12-month period using the previously validated Australian *Salmonella* spp. mix-ELISA (van der Heijden, 2001). The ten herds ranged in production capacity from 80 to 1500 sows and were selected to maximise the opportunity for expression of the impact of transport distance. Five herds were situated < 125 km (short-haul) and five > 500 km (long-haul) from the abattoir. On four occasions (seasonally) each farm withdrew feed from three groups of 20 slaughter pigs so they were off feed for 12-18 hours, 18-24 hours and >24 hours prior to slaughter: includes time-off-feed on farm before transport, transport time and lairage time; (10 herds x 4 seasons x 3 treatments = 120 batches of 20 pigs). Therefore, the three time-off-feed treatments were measured within each testing round within each herd. This design is highly powered for the comparison of the three time-off-feed treatments within each transport scenario, which may otherwise be collinear in an observational study. Blood and caecal ingesta (10 g /pig) were collected at slaughter for serology and culture (Australian Standard Method AS1766.2.5-1991) respectively, with results correlated on a batch, not individual pig basis.

Statistical methods: The experimental design resulted in a 3-level nested structure in the data. Therefore, effects of time-off-feed treatment, transport distance and serological prevalence on caecal positivity were analysed as a 3-level hierarchical linear model (HLM for Windows, Version 5.04) using the logit link at level 1 and a penalised quasi-likelihood (PQL) estimation method. Average serological prevalence (centred around the mean of 35 %) was measured for each of the three time-off-feed treatments for each batch (testing round) of pigs within each herd (level 1 covariates). Four batches of pigs were tested within each herd (level 2, intercept only), and transport distance was a between herd (level 3) covariate. All intercept terms were treated as random; the remaining parameters were treated as fixed effects. Odds ratios were estimated from the population-averaged (as opposed to unit-specific) regression parameters.

Results

Batch serology and culture results: There was no significant difference in the serology profile between 125 km and 500 km herds ($p = 0.352$). Overall 700 (35.4 %) of 1977 blood samples from the 10 study herds were positive in the seasonal sampling rounds. The sero-positive range on a batch basis was 0-100 %. The average culture positive rate over all herds was 5.3 % (2.8, 9.9; 95 % CI), which gave an average sero-prevalence to caecal prevalence ratio of 6.7:1. The culture positive range on a batch basis was 0-70 %.

Effect of time-off-feed and transport distance: There was a significant reduction in caecal culture positivity in the < 125 km (short-haul) herds with increasing time-off-feed prior to slaughter (Table 1). The converse was true for the > 500 km (long-haul) herds, where a significant increase was seen with increasing time-off-feed. There was a trend towards a positive correlation between culture positive rate and serological prevalence, which did not reach statistical significance [$p = 0.114$ (data not shown)]. When serological prevalence was excluded as a covariate, the effects of time-off-feed and transport distance were essentially unaltered.

Table 1. Association of distance to abattoir and time-off-feed before slaughter with *Salmonella* spp. caecal culture positivity for 10 herds situated either < 125 km or > 500 km from abattoir.

| Off-feed Effect | Odds Ratio (95%CI) | p-value | Fitted Prevalence ¹ (%) (95%CI) |
|--------------------------|-----------------------|---------|---|
| < 125 km Herds | | | |
| 12-18 hours off feed | 1.0 | | 11.0 (4.0, 26.7) |
| 18-24 hours off feed | 0.33 (0.18, 0.59) | <0.001 | 3.9 (1.3, 11.2) |
| > 24 hours off feed | 0.20 (0.10, 0.39) | <0.001 | 2.4 (0.7, 7.4) |
| > 500 km Herds | | | |
| 12-18 hours off feed | 1.0 | | 3.0 (1.0, 8.8) |
| 18-24 hours off feed | 1.44 (0.73, 2.84) | 0.288 | 4.3 (1.5, 12.0) |
| > 24 hours off feed | 2.07 (1.10, 3.89) | 0.023 | 6.1 (2.2, 15.8) |

1. Prevalences were estimated by back-transformation of the logits from the regression mode (with the serology covariate excluded)

Salmonella serovars: In total there were 109 isolates from 1871 caecal samples. Of these, 52 (48 %) were *S. Derby* and 25 (23 %) *S. Infantis*. Only seven (6 %) of the isolations were *S. Typhimurium*.

Discussion: Regulatory programs have stopped short of mandating specific on-farm control procedures due to inadequate knowledge of the epidemiology of foodborne infections in animal populations. This study adds to the understanding of this complex issue by proposing two models based on the combined effect of time-off-feed and transport distance (Table 1) that may explain some of the apparent contradictions in the literature regarding *Salmonella* spp. infection in slaughter pigs.

Model 1-short haul: For herds < 125 km from the abattoirs, *Salmonella* spp. infection was found to decrease significantly with an increase in the period between last feed and slaughter. In this model, fasting is a protective factor offsetting any increase in shedding caused by (short distance) transport stress as found by Williams and Newell (1970) and Isaacson *et al* (1999 a).

Model 2-long haul: For herds > 500 km from the abattoirs, the protective effect of fasting is apparently overridden by other factors. There is a significant increase in *Salmonella* spp. infection with an increase in the period between last feed and slaughter, which is consistent with the findings of Williams and Newell (1967) and Isaacson *et al* (1999 b).

Thus time-off-feed and transport distance appeared to strongly influence caecal positivity at slaughter. However, herd sero-prevalence did not exert a significant effect in this study, indicating that a national serological monitoring programme would have limited application under the Australian marketing scenarios investigated.

The Australian Pork Industry Quality Standards presently advise a fasting period of 'at least 6 hours' before slaughter to minimise PSE and gut spillage during processing and slaughtering and 'no more than 24 hours' off feed to minimise *Salmonella* spp. build up. The findings in this study suggest tightening of the pre-slaughter time-off-feed period to 15 to 24 hours, which should include a period of fasting prior to transport where possible, as the best approach to minimising *Salmonella* spp. in ingesta of slaughter pigs.

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Macrolide resistance in porcine streptococci: a human health hazard?

O 65

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Summary: In order to obtain better insights into the possible exchange of resistance genes between human and porcine streptococci, macrolide and lincosamide resistant streptococci from tonsillar and colon swabs from pigs and pork carcass swabs were isolated and their resistance phenotypes and genotypes were determined. The sequences of the *erm(B)* genes of 21 human streptococci, 22 porcine streptococci and 15 streptococci isolated from pork carcasses were compared. From each of the 33 pigs and from 88 of 99 carcass swabs, at least one resistant streptococcal strain was isolated. The predominant phenotype was the constitutively expressed MLS_B phenotype, mostly encoded by the *erm(B)* gene. Identical *erm(B)* gene sequences were present in strains from humans, pigs and pork carcasses.

Keywords: Macrolide resistance, *erm(B)*, streptococci, swine, resistance transfer

Introduction: Two types of antimicrobial drug resistance transfer from animals to humans might be of importance. The direct way of resistance transfer occurs when resistant zoonotic bacteria infect humans. The contribution of the indirect way of resistance transfer to the antimicrobial resistance problems in human medicine is less clear. Indirect way means that resistance genes from bacteria associated with animals are transferred to bacteria associated with humans. One of the most important antibiotic groups affected by resistance in humans and in pigs is the macrolide-lincosamide-streptogramin B (MLS_B) antibiotic family. The present study was carried out in order to obtain better insights in the possible exchange of resistance genes between human and porcine streptococci.

Materials and methods: Tonsillar and colon swabs were collected from 33 pigs, originating from 33 different farms in Belgium. Ninety-nine swabs were taken by swabbing the entire skin surface of pork carcasses immediately after slaughter in four slaughterhouses. The pigs originated from 35 different

Belgian farms. The swabs were inoculated on Columbia agar (Oxoid, England) supplemented with 5% sheep blood, colistin and aztreonam, supplemented with 1 µg/ml erythromycin, 8 µg/ml erythromycin or 10 µg/ml lincomycin. All colony types were purified and identified using rDNA-PCR (Baele et al., 2001). From each animal or carcass only one strain per species carrying the same resistance phenotype and genotype was included in the results. Antimicrobial susceptibility patterns of the strains were determined by disk diffusion on Columbia blood agar using the antimicrobial test tablets clindamycin, erythromycin, lincomycin and tylosin. An MLS_B phenotype isolate was defined as an isolate resistant to erythromycin, clindamycin, lincomycin and tylosin. An M phenotype isolate was an isolate resistant to erythromycin alone, and the L phenotype showed resistance to lincosamides only. The presence of the *erm(B)* and *mef(A)* genes was determined by PCR using primers derived from published sequences (Martel et al., 2001; Sutcliffe et al., 1996). The sequences of the *erm(B)* genes of 21 human streptococci (two *S. oralis-mitis* sp., eight *S. pneumoniae*, five *S. pyogenes*, one *S. salivarius*, three *S. sanguinus* and two *S. thermophilus* strains), 22 porcine streptococci (three *S. alactolyticus*, one *S. bovis*, one *S. dysgalactiae*, five *S. gallolyticus*, three *S. hyointestinalis* and nine *S. suis* strains) and 15 streptococci isolated from pork carcasses (two *S. alactolyticus*, one *S. bovis*, two *S. dysgalactiae*, two *S. hyointestinalis*, three *S. pneumoniae* and five *S. suis* strains) were determined using the BigDye Terminator Cycle Sequencing kit (PE Biosystems) and the primers 5'ATGAACAAAATATAAAATATT3' and 5'TTATTTCCTC CCGTAAA3'.

Results: From tonsillar and colon swabs from each of the 33 pigs and from 88 out of the 99 pork carcass swabs at least one resistant streptococcal species was isolated. Their resistance phenotypes and genotypes are presented in Tables 1 and 2. The MLS_B phenotype was most frequently detected and generally encoded by *erm(B)* genes. Sequencing of these genes from different streptococci showed a similarity between 98.7% and 100%. Identical *erm(B)* genes were present in streptococcal strains isolated from humans, pigs and pork carcasses.

Table 1. Resistance phenotype and genotype from streptococci isolated from tonsillar and colon swabs from 33 pigs.

| | Isolated from | Number | Number MLS _B * | Number L* | Number <i>erm(B)</i> + | Number <i>mef(A)</i> + |
|--------------------------------------|---------------|--------|---------------------------|-----------|------------------------|------------------------|
| <i>Streptococcus alactolyticus</i> | C | 9 | 9 | | 9 | 0 |
| <i>Streptococcus bovis</i> | C | 4 | 2 | 2 | 2 | 0 |
| <i>Streptococcus dysgalactiae</i> | T | 1 | 1 | | 1 | 0 |
| <i>Streptococcus gallolyticus</i> | C | 8 | 8 | | 8 | 0 |
| <i>Streptococcus hyointestinalis</i> | C, T | 4 | 4 | | 4 | 0 |
| <i>Streptococcus pluranimalium</i> | T | 1 | 1 | | 1 | 0 |
| <i>Streptococcus suis</i> | C, T | 33 | 33 | | 32 | 1 |

C Colon swab, T Tonsillar swab, * resistance phenotype

Table 2. Resistance phenotype and genotype from streptococci isolated from 99 pork carcasses.

| | Number | Number MLS _B * | Number M* | Number L* | Number <i>erm(B)</i> + | Number <i>mef(A)</i> + |
|--------------------------------------|--------|---------------------------|-----------|-----------|------------------------|------------------------|
| <i>Streptococcus agalactiae</i> | 1 | 1 | | | 1 | 0 |
| <i>Streptococcus alactolyticus</i> | 42 | 34 | | 8 | 33 | 1 ^a |
| <i>Streptococcus bovis</i> | 7 | 7 | | | 7 | 0 |
| <i>Streptococcus dysgalactiae</i> | 23 | 15 | 5 | 2 | 13 | 8 (1) |
| <i>Streptococcus hyointestinalis</i> | 11 | 11 | | | 8 | 2 |
| <i>Streptococcus pneumoniae</i> | 4 | 4 | | | 3 | 1 |
| <i>Streptococcus suis</i> | 43 | 41 | 2 | | 40 | 3 (1) |
| <i>Streptococcus hyovaginalis</i> | 2 | 1 | | 1 | 1 | 0 |
| <i>Streptococcus porcinus</i> | 3 | 2 | | 1 | 2 | 0 |

* resistance phenotype, ^a strains with MLS_B phenotype carrying only the *mef(A)* gene

Discussion: In this study, macrolide resistant streptococci were frequently found in tonsillar and colon swabs from pigs and on pork carcasses. The predominant resistance phenotype was the MLS_B phenotype. A minority of the strains showed the M-, L- and ML phenotype. A similar distribution of phenotype patterns was obtained by Lagrou et al. (2000) with Belgian *S. pneumoniae* isolates. The MLS_B phenotype was found to be encoded mainly by the *erm(B)* gene. In human streptococci in Belgium, *erm(B)* encoded resistance is also the most important mechanism in *S. pneumoniae* and *S. pyogenes* (Descheemaeker et al., 2000; Lagrou et al., 2000). Since identical *erm(B)* genes were found in porcine and in human strains, it might be possible that this gene is transferred between animal and human strains. Further studies are required to obtain better insights into possible exchange of resistance genes between human and porcine streptococcal strains. These studies should include identification of mobile DNA elements in human and porcine strains. Localisation of identical *erm(B)* genes on identical plasmids or transposons would be a further indication of transfer of these genes between these strains.

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Adaptive resistance to Biocides and implications of cross-resistance to Antimicrobial Agents in Foodborne Pathogens. O 66

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Summary: This study was focused on the potential for adaptive resistance in *Salmonella* and *Escherichia coli* to commonly used biocides, to identify resistance strategies and any cross-resistance to antibiotics. Bacteria were serially exposed in sub-inhibitory concentrations of biocides and adaptive resistance was observed in all strains investigated. Erythromycin-resistant *Salm.* Enteritidis did not cross-resist to biocides, whereas erythromycin-resistant *Salm.* Typhimurium express cross-resistance to chlorohexidine. Benzalkonium chloride-resistant *Salm.* Virchow showed an elevated resistance to chlorohexidine, however chlorohexidine-resistant *Salm.* Virchow did not demonstrate it back. Triclosan-

resistant *E. coli* O157 strains exerted decreased susceptibility to chloramphenicol, erythromycin, imipenem, tetracycline and trimethoprim and to biocides. Conversely, TLN-adapted *E. coli* O55 and K-12 did not show any cross-resistance to the antimicrobial agents tested. Possibly, domestic kitchens and places of commercial food production may provide a selective environment for bacterial adaptation, which may lead to the undesirable situation of resident strains becoming resistant and cross-resistant to other antimicrobials.

Keywords: *Salmonella* Typhimurium, *Escherichia coli* O157, Erythromycin, Chlorohexidine, Triclosan.

Introduction: Currently, pork producers are facing many challenges including food safety, pork quality and welfare standards. Antimicrobial use and resistance of pathogens associated with pork is another very important issue. There is currently much interest regarding drug administration in animals, especially with the link to the emergence of multiple antimicrobial resistant zoonotic bacterial pathogens. Research on the antimicrobial resistance of foodborne pathogens is becoming increasingly critical due to increased awareness and public concern over bacterial food safety. Thus, this study was focused on the mechanisms underlying antimicrobial resistance in *Salmonella* and *E. coli*. This may help in the future design of new antimicrobial preparations, which will help to overcome existing issues.

Materials and Methods: Antimicrobial agents and Biocides. Antimicrobial agent disks included amoxicillin (AMX), amoxicillin/clavulanic acid (AMC), chloramphenicol (C), ciprofloxacin (CIP), clindamycin (CL), colistin sulfate (CS), fusidic acid (FD), gentamycin (GEN), imipenem (IPM), rifampicin (RIF), tetracycline (TET), trimethoprim (TMP), vancomycin (VAN), and erythromycin (ERY). Biocides included benzalkonium chloride (BKC), chlorohexidine (CHX) and triclosan (TLN).

Bacterial Identification: Random Amplification of Polymorphic DNA was employed to confirm strain continuity (Hopkins and Hilton, 2001).

Minimum Inhibitory Concentration (MIC) and Serial Passage: The MIC was determined using a standard broth dilution method carried out using a two-fold dilution of each antibacterial agent (Loughlin et al., 2002). Bacterial adaptation was performed following the technique described by Joynson et al., 2002.

Cross-resistance to Antimicrobial Agents & Biocides: Cross-resistance towards various antibiotics and biocides was determined using the Stokes' method (Anon. 1991).

Results: **Bacterial Identification:** Following each passage, pre-and post-adapted strains were characterised by RAPD profiling to ensure strain continuity (Fig.1). All strains shared the same RAPD profile.

MICs: The gradual passage of *Salmonella enterica* and *Escherichia coli* strains to sub-inhibitory concentrations of ERY, BKC, CHX and TLN, produced cultures capable of growth at high concentrations (Table 1). Those cultures capable to grow at the maximum concentrations of the antibacterials tested were used for the performance of the cross-resistance experiments.

Cross-resistance to Antimicrobial Agents & Biocides: The increase in the MICs through adaptation to antimicrobials tested, did confer cross-resistance in some instances. Triclosan-adapted *Escherichia coli* O157 expressed decreased susceptibility to a number of antibiotics, including chloramphenicol, erythromycin, imipenem, tetracycline, trimethoprim and to various biocides (Fig. 2), whereas other *E. coli* strains did not. *Salmonella enterica* strains did not generally show decreased susceptibility to antibiotics/biocides with the exception of *Salm.* Typhimurium and *Salm.* Virchow, which did in some instances. Generally though, in TLN-adapted *Salmonella enterica* cross-resistance between antibiotics and biocides did not occur frequently.

Figure 1: RAPD from TLN-adapted K-12 strains. Lanes 1 to 4 represent no. of passages. M is a 1 kbp molecular weight marker.

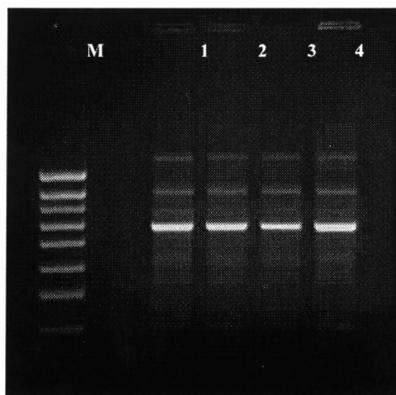
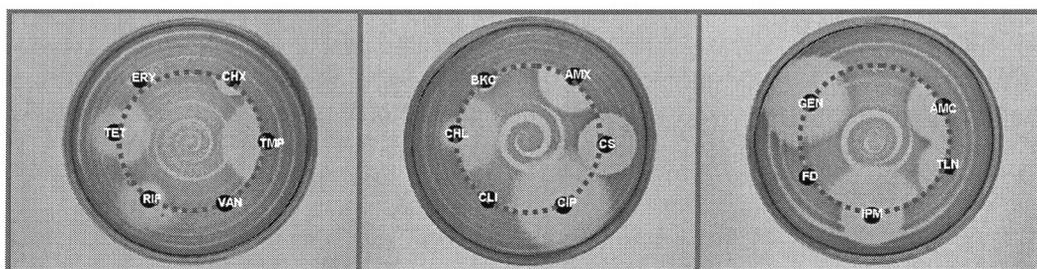


Figure 2: Cross-resistance between TLN pre- & post-adapted *E. coli* O157 strains.



| Strains | ERY MIC(mg/L) | BKC MIC(mg/L) | CHX MIC(mg/L) | TLN MIC(mg/L) |
|---|------------------|------------------|------------------|------------------|
| <i>Salm. Enteritidis</i> Parent / Adapted | 256 / 2048 | 32 / 256 | - | 16 / 512 |
| <i>Salm. Typhimurium</i> Parent / Adapted | 256 / 512 | 32 / 64 | - | 8 / 512 |
| <i>Salm. Virchow</i> Parent / Adapted | 32 / 512 | 4 / 256 | 8 / 128 | 16 / 1024 |
| <i>E. coli</i> O157 Parent / Adapted | 256 / 1024 | 16 / 1024 | 4 / 512 | 0.25 / 2048 |
| <i>E. coli</i> O55 Parent / Adapted | - | - | - | 1 / 1024 |
| <i>E. coli</i> K-12 Parent / Adapted | - | - | - | 0.125 / 1024 |

Table 1: Summary of Susceptibility of pre- & post-adapted *Salmonella enterica* and *E. coli* strains to antimicrobials.

Discussion: Cross-resistance to different antibacterial agents including quinolones and nalidixic acid, chloramphenicol, trimethoprim and in some cases b-lactam antibiotics is a common phenomenon in Gram-negative bacteria (Gutmann et al., 1995). In this study adaptive resistance in *Salmonella enterica* and *E. coli* O157, O55 and K-12 was readily achieved by passage in sublethal concentrations of antibacterial agents, which conferred cross-resistance to other antibiotics and biocides. Interestingly, adaptive resistance to TLN by *E. coli* O157 appeared to confer a marked increased sensitivity to AMC, AMX and IPM and to a lesser degree to CHL, CS and GEN. Differences between the adaptive- and cross-resistance profiles between K-12, O55 and O157 suggest that strain-specific rather than global mechanisms are underlying the resistance observed, some of which may be facilitated by the additional genes O157 is known to possess over K-12 and potentially O55 (Perna et al., 2001). No obvious correlation could be drawn between *Salmonella* serotype and resistance to a particular class of antibiotics or group of biocides, however, this does not detract from the finding that in particular strain / antibiotic / biocide combination strong evidence of cross-resistance was observed.

Conclusions: These findings support the concern that repeated sub-lethal exposure to biocides not only promotes adaptive resistance but also confers a decreased sensitivity to antibiotics.

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TETRACYCLINE RESISTANCE GENES IN SALMONELLA FROM GROWING PIGS AND THEIR RELATIONSHIP TO ANTIMICROBIAL USE AND RESISTANCE TO OTHER ANTIMICROBIALS.

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Summary: The aim of this study was to describe the occurrence of three genes coding for tetracycline resistance in *Salmonellae* isolated from normal slaughter weight pigs, and to test for relationships between the occurrence of these genes, phenotypic resistance, and the use of antimicrobials in feed and water. *Salmonella* (1,431) were cultured at slaughter or just before slaughter among slaughter-age pigs, and were isolated using conventional methods. Three tetracycline resistance genes were

tested using denaturing gel electrophoresis (DGGE). Phenotypic tetracycline resistance was observed as the phenotype of 52.0% of isolates. Tetracycline resistance genes, designated A, B and C, were detected in 18.6%, 66.4% and 1.6% of isolates, respectively. When broken down by genetic pattern, resistance/intermediate resistance was found in the following proportion of isolates: A-,B-,C-, 23.0%; A+, B-, C-, 93.1%; A+, B-, C+, 94.7%; and A-, B-, C+, 69.7%. Reported use of antibiotics in feed or water was not significantly correlated with phenotypic resistance or the occurrence of any of the three genes studied. The *tet(C)* gene was positively associated with phenotypic resistance to tetracycline, sulphamethoxazole, streptomycin, ceftiofur, kanamycin and cephalothin. It is concluded that antimicrobial resistance to tetracyclines in growing pigs is common, that the *tet(C)* gene is common, that *tet(C)* is associated with phenotypic resistance to multiple antimicrobials, and that current antimicrobial use is not an important predictor of tetracycline resistance genes.

Keywords: antimicrobials, swine, bacterial genetics

Introduction: Antimicrobial resistance is important for Salmonellae since foodborne illness can have severe health consequences in humans with resistant infections with resistant infections. Understanding the epidemiology of tetracycline resistance in swine isolates can help us understand antimicrobial resistance of other antibiotics that are more crucial in human health. Further, genes coding for resistance to tetracyclines may be coupled to genes coding for resistance to other antibiotics. We designed this study to investigate the occurrence of tetracycline resistance genes, and to examine relationships between these genes and both use of antimicrobials on commercial pig farms and to the expression of resistance to other antimicrobials.

Methods and materials: Herds with a history of delivering at least 30 animals per shipment were solicited from among those supplying slaughter weight pigs to two Midwestern U.S. abattoirs. Among 328 farms solicited, 205 agreed to participate, and 141 were selected based on convenience of scheduling. Farms were collected one to five times over a three year period. After slaughter, 10 g caudal mesenteric lymph node tissue was aseptically collected from 30 randomly selected pigs. In addition, from 30 herds a 10g fecal sample was collected at the farm less than 48 hours prior to shipment to slaughter.

Conventional bacterial culture methods were used to isolate *Salmonella* from all samples, using a slight modification of a procedure previously described. [Fedorka-Cray et al., 1998] For the first sample from each farm only, five samples were pooled, combining two grams from each pig. Samples were blended and incubated in tetrathionate broth for 48 hrs at 37°C. One ml of this broth was transferred to R-10 broth and incubated 24 hrs at 37°C. XLT-4 plates were streaked for isolation, followed by culture on Brilliant Green agar, and finally suspect colonies were tested for agglutination with polyvalent anti-*Salmonella* sera. From positive pools, retained frozen tissue was cultured individually using two-gram samples in 20 ml of tetrathionate. This same individual procedure, without freezing, was used for subsequent samples collected.

Approximate MIC values were determined for 17 antimicrobials using the Sensititre™ system (TREK Diagnostic Systems, Inc., Cleveland, Ohio, U.S.A.). The antimicrobials were amikacin, amoxicillin/clavulanic acid, ampicillin, apramycin, cefoxitin, ceftiofur, ceftriaxone, cephalothin, chloramphenicol, ciprofloxacin, gentamicin, kanamycin, nalidixic acid, streptomycin, sulphamethoxazole, tetracycline, and trimethoprim/sulphamethoxazole. Procedures described by the manufacturer were followed. Interpretation of results were as specified by the manufacturer, except that both intermediate and resistant outcomes were considered resistant for purposes of further analysis.

The isolates were tested for the presence of three tetracycline efflux pump genes, termed *tet(A)*, *tet(B)* and *tet(C)*. PCR based methods were used to identify these genes [Aminov, R.I., et al.]. Farm managers were asked, by a written survey, to describe the use of antimicrobials in the group of pigs

marketed. They described which antimicrobials were used, at what dose, at what age, and whether the drug was used for therapy or for growth promotion. Relationships between gene presence and tetracycline phenotype, and relationships between antimicrobial use and *tet(C)* were assessed by logistic regression, adjusting for the clustering of samples within herd. (Egret, Cytel Software, Inc., Cambridge, Massachusetts, U.S.A). The relationships between *tet(C)* to phenotypes to other antimicrobials were assessed by the Chi-Square statistic.

Results: A total of 1431 *Salmonella* isolates were tested. Tetracycline resistance was observed in 50.9% of isolates, while 1.4% were intermediate in sensitivity. The *tet(A)*, *tet(B)* and *tet(C)* genes were detected in 18.6%, 1.6% and 66.4% of isolates, respectively. When broken down by genetic pattern, resistance / intermediate resistance was found in the following proportion of isolates: A-,B-,C-, 23.0%; A+, B-, C-, 93.1%; A+, B-, C+, 94.7%; and A-, B-, C+, 69.7%. The percentage of bacteria with resistant phenotypes, but for which no resistance gene was found was 6.2%. The percentage of isolates with one or more gene detected but with susceptible phenotypes was 29.4%. Because of the small proportion of isolates with *tet(B)* detected, further analysis was not conducted for this gene. The odds of expressing the tetracycline resistance phenotype were higher for isolates with the *tet(A)* (OR = 15.4) and *tet(C)* (OR = 3.8) genes, when compared to isolates where the genes were not detected. There was no statistical evidence of interaction between the genes.

Antimicrobial phenotypic resistance to seven antimicrobials was associated with the detection of *tet(C)*. The antimicrobials and the odds ratio of association for each were sulphamethoxazole (12.7), tetracycline (11.2), streptomycin (6.8), ceftiofur (3.3), kanamycin (2.1), cephalothin (1.9) and chloramphenicol (0.4).

Antimicrobials used in feed or water during the finishing phase by more than 10% of herds were tetracyclines, bacitracin methylene disalicylate and tylosin. No statistical relationship was detected between the use of these antimicrobials and *tet(C)*.

Discussion: The lack of association between antimicrobial use and the most commonly detected tetracycline resistance genes suggests that tetracycline resistance, one acquired, does not rapidly resolve. The historical usage patterns on these farms was not described, so it is not known how long it had been since an antibiotic was used on a farm. However, tetracyclines were reported to be commonly used. It is possible that farms which at the time of the study were not using tetracycline may have used them in the past.

These genes confer the resistance due to the efflux of tetracycline from the cell catalyzed by drug:H⁺ antiport. [Paulsen IT, et al.] The genes studied here were a sufficient explanation for the majority of the phenotypically tetracycline resistant detected, although a minority of resistant isolates had none of the genes. Conversely, a relatively large proportion of bacteria carried one of the resistance genes, but did not express resistance *in vitro*. The assay used detects genetic fragments from the genes in question, but does not test whether the genes are activated and/or effective. It appears that in some cases these genes are not capable of effectively activating the efflux pumps, or some additional factor effectively renders the pumps ineffective.

We conclude a small number of genes can account for the majority of tetracycline resistance observed on these commercial pig farms, that tetracycline genes remain in the pool of *Salmonella* strains on farms after the withdrawal of the tetracyclines from the diet, and that one gene, *tet(C)*, is associated with resistance other antimicrobials, directly or indirectly.

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Reported antimicrobial use and *Salmonella* resistance on 90 Alberta swine farms

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Summary: The study objectives were to describe antimicrobial use (AMU) and *Salmonella* resistance on 90 Alberta swine farms. The vast majority of antimicrobials were used in-feed. In weaners, in-feed use did not vary among farms, suggesting heavy reliance on in-feed antimicrobials. For grow-to-finish production phases, most farms reported heavy reliance on in-feed antimicrobials, but 6 and 14 farms did not report any in-feed AMU in growers and finishers, respectively. The tetracycline-sulphamethazine-penicillin combination and carbadox were the most common antimicrobials added to the weaner rations, while tylosin and lincomycin were the most common antimicrobials added to grower and finisher rations. No resistance was observed to nalidixic acid, ciprofloxacin, amikacin and ceftiofur. A low frequency of resistance (<5%) was observed to gentamicin, apramycin, cephalotin, ceftiofur, amoxicillin/clavulanic acid and trimethoprim-sulphamethoxazole. Most common resistances were detected to tetracycline, streptomycin, sulphamethoxazole, kanamycin and ampicillin. Despite widespread AMU, 40.19% of *Salmonella* isolates were susceptible to 17 antimicrobials.

Keywords: antimicrobial drugs, susceptibility, *Salmonella* serotypes

Background: The emergence of antimicrobial resistance (AMR) is believed to be associated with the use of antimicrobial drugs in human medicine, veterinary medicine and food animal production. The scope and magnitude of the public health impact of antimicrobial use (AMU) in animals remains unclear since there is relatively little information on AMU and the prevalence of resistant bacteria in food animals (McEwen & Fedorka-Cray, 2002). The objectives of this study were to describe AMU and *Salmonella* resistance on 90 Alberta swine farms.

Materials and Methods: Ten swine veterinarians selected 90 Alberta swine farms. AMU data were gathered through a questionnaire, which was completed by the owner or operator of the farm along with the herd veterinarian. Fifteen fecal samples and five environmental samples per farm were collected over a four-month period from the finishing swine and the farm environment. All samples were tested for *Salmonella* using bacteriological culture. *Salmonella* isolates were serotyped by the Health Canada O.I.E Reference Laboratory for Salmonellosis (Guelph, Ontario). Susceptibility testing was performed on all isolates using a Sensiitre Custom MIC Panel (Trek Diagnostic Systems Ltd.).

Results: Selected farms represented approximately 25% of the Alberta annual market pig production. Eleven AMU farm patterns were reported. Among 78 farrow-to-finish farms, 45 farms reported use of in-feed antimicrobials more than 95% of the time in the weaner, grower and finisher rations. Seven farms reported use of in-feed antimicrobials more than 95% of the time in the weaner and grower rations and 50-95% the time in the finisher rations. Eight farms reported use of in-feed antimicrobials more than 95% of the time in the weaner and grower rations and 0% of the time in the finisher rations. Six farms reported use of in-feed antimicrobials more than 95% of the time in the weaner rations, and 0% of the time in grower and finisher rations. The tetracycline-sulphamethazine-penicillin combination and carbadox were the most common antimicrobials added to the weaner rations. Tylosin and lincomycin were the most common antimicrobials added to grower and finisher rations. Mainly occasional AMU in water was reported in weaners, growers and finishers. Penicillin and tetracycline were the most common antimicrobials added to water for all three production categories.

At least one *Salmonella* isolate was recovered from 60 of 90 participating farms. Among 418 *Salmonella* isolates, 40.19% of isolates were susceptible to all antimicrobials. No resistance was observed to nalidixic acid, ciprofloxacin, amikacin and ceftriaxone. A low frequency of resistance (<5%) was observed to gentamicin, apramycin, cephalotin, ceftiofur, amoxicillin/clavulanic acid and trimethoprim-sulphamethoxazole. Most common resistances were detected to tetracycline (44.3%), streptomycin (29.7%), sulphamethoxazole (25.4%), kanamycin (15.1%) and ampicillin (10.4%). Among 418 isolates, 67 (16.0%) were resistant to 4 or more antimicrobials. *Salmonella Derby*, *Typhimurium* and *California* were the most resistant serotypes.

Discussion and Implications: On most farms the vast majority of antimicrobials were used in-feed. Reported in-feed AMU patterns in weaners did not vary among farms, suggesting heavy reliance on in-feed antimicrobials at this stage of production. Similar findings have been recently reported in USA (Bush & LeRoy-Biehl, 2002). In growers and particularly in finishers reported in-feed AMU patterns varied among farms. Some farms reported heavy reliance on in-feed antimicrobials in these stages of production, but 6 and 14 farms did not report any use of in-feed antimicrobials in growers and finishers, respectively. Most common resistances were detected to tetracycline, streptomycin, sulphamethoxazole, kanamycin and ampicillin. These antimicrobials have been extensively used in swine production and medicine for decades. Levels of most common resistances observed in our study were relatively moderate (<50%) compared to some USA studies (Wondwossen et al., 2000; Farrington et al., 2001). These studies reported higher levels of resistance to these antimicrobials, particularly for tetracycline (>80%). A moderate level of resistance (16.9%) was observed for chloramphenicol, an antimicrobial not been used in veterinary medicine for decades, suggesting a genetic linkage between this resistance and resistance to other antimicrobials. Despite widespread reported AMU, 40.2% of *Salmonella* isolates were susceptible to all antimicrobials. Further analysis of data may improve the understanding of the relationship between AMU and *Salmonella* resistance. The information obtained from this study might provide valuable surveillance information for appropriate and sound decisions regarding prudent AMU practices in swine, and future courses of action relating to AMU and AMR issues.

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VetStat – The Danish nation-wide monitoring of veterinary medicine use on herd level

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Summary: The Danish monitoring programme, VetStat, collects detailed data on all veterinary usage of therapeutic drugs and coccidiostats on herd level. The detailed information enables standardization of drug usage and allows comparison between individual herds. The vision is to prevent misuse and optimize the usage of antimicrobial drugs. This is achieved by providing information to farmers, veterinary practitioners, and control authorities about usage patterns and linking with bacterial antimicrobial susceptibility data at farm level, and by aiding the development of prudent guidelines. Data will be used in research and in risk assessment to aid the development of risk based control strategies in the food chain, reducing the spread of resistant bacterial infections from animals to humans. The presentation gives a description of the design of the system, the validation of the data, and the prospects for using the information to protect animal and public health.

Keywords: antimicrobial, resistance, surveillance, drug statistics, veterinary.

Introduction: In recent years, a global concern of the veterinary antimicrobial use contributing to antimicrobial resistance both in animals and humans has arisen. Detailed information on utilization of antimicrobial drugs in food animals is vital for assessment of the contribution of antimicrobial usage to the emergence and trends in resistance problems and for the evaluation of the effect of the measures taken. Acting on the recommendations from "The Microbial Threat", an invitational EU conference held in Copenhagen in 1998, the Danish government decided to implement herd level monitoring of all prescription medicine usage in production animals. While Denmark had for some time monitored such usage on the basis of information provided from the top of the distribution system (i.e., by the pharmaceutical industry) the new monitoring program, VetStat, collects data close to the end user. Since 2001, VetStat has produced valid data at the national level and monthly usage statistics are published at the Danish Zoonosis Centre homepage (<http://vetstat.vetinst.dk>).

Data collection. In Denmark, virtually all therapeutic medicines are prescription-only and, with few exceptions, available only through pharmacies. Medicines obtained by veterinarians for use in practice or for re-sale to clients must also be purchased through pharmacies. The only exceptions to the pharmacy monopoly are vaccines, and the use of premixes approved for use in medicated feed at licensed feed mills. The medicated feed is obtained by the farmer only on the basis of a veterinary prescription. However, coccidiostats approved in the EU as feed additives are freely available from the feed mills. More than 95% of the antimicrobial drugs used in pig production are sold directly to the farmer from the pharmacy, while about 2% is purchased at the feed mills.

The VetStat program collects data from feed mills, production animal practice, and pharmacies on feed additives (coccidiostats and antimicrobial growth promoters) and all prescription medicines, including both veterinary and human medicines used in animals. The data record, describing each prescription item used or sold to the farmer, comprises

- Date (of sales if not of usage by veterinarian)
- Source: Identity of dispensing pharmacy, feed mill, or the veterinarian using the drug.
- Prescriber: Identity code of veterinarian and identity code of the practice.
- Drug information: Quantity dispensed and a numerical code identifying unequivocally the type of medicine, including the formulation, active components, strength and size of pack, administration route and ATC/ATCvet -code (Anonymous,1999).

• Recipient: Farm identity code (CHR-ID), intended animal species, age group (when appropriate), and disease category. In poultry, the "age-group" represents the animal production classes. For medicines sold at pharmacies for use in practice, similar drug information, the date, and the receiving practice is identified. Data on companion animals and horses are reported with fewer details.

At the pharmacies, the information is extracted automatically during electronic processing of sales, and data are transferred at least once a month to VetStat. Feed mills records on sales are entered directly to the database via the Internet, or transferred once a month. Veterinary practitioners report to all prescription medicines used in practice for treatment of production animals. For data collection, most practices use software that automatically extracts the information required by VetStat during the billing procedure. Veterinarians also have the option of recording usage directly into the VetStat database via the Internet.

Data validation. The VetStat information is subject both to logic validation on some data and to validation of the amount of drugs reported. Logic validation is a correctional "filter" where all information has to comply with certain criteria contained in fixed table within the database. Data from pharmacies are subject to logical validation of the Nordic item number at data entry, and subsequent validation of the correspondence between animal code, age group, and CHR number after data entry. The percentage of errors is low and declining (In 2002, 4%-7% erroneous codes) and the sources of errors have currently been addressed. Data from the feed mills are subject to logic validation of the individual codes at data entry over the Internet. The percentage of errors in the data from the veterinarians has been high due to technical difficulties in the complex system with multiple routes for transferring data from the veterinary practitioners. A number of technical errors have been corrected in 2002 and, from the onset of 2003; the data are subject to full logic validation within the database. Data records with non-corresponding data on age group, animal species and disease classes, invalid CHR number, or invalid constellations of drug identity and units are returned the practitioner, who must then correct the data via the Internet. Validation of the amounts of drugs is facilitated by the fact that virtually all therapeutic drugs are prescription-only. The data on amounts of medicines used in veterinary practice may be validated against pharmacy data on sales to the specific practice. Pharmacy and feed mill data on annual usage of antimicrobial drugs have been validated against the wholesalers' statistics, and the VetStat data on total antimicrobial usage have been found to be reliable (Anonymous, 2002). The pharmacy information on drug identity and amounts has a very high validity due to a strict relation to the electronic registration of any sale conducted at the pharmacies.

The database. The VetStat database is a relational database on an Oracle platform and is part of the so-called GLR/CHR register, operated on behalf of the Ministry of Food, Agriculture and Fisheries. This GLR/CHR comprise also the central husbandry register (CHR), containing information on farm level about the animal species the number of animals within different age groups, production type, geographic information among other information.

The detailed information in VetStat enables standardization of drug usage, taking into account the potency of the drugs and the animals in which they are used, providing better measures of antimicrobial usage on a national level, and allowing comparison between individual herds. Standardized animal daily doses (ADD) have been defined for every therapeutic formulation (each Nordic item number) and each species. The general principles for standardization of dosage for animals are parallel to the defined daily dosage (DDD) used for human usage, i.e., the mean daily maintenance dose for therapeutic use for the main indication, required to treat an animal of a certain species. For each species and age group, a standard animal weight has been defined, to enable the calculation of an ADD. By relating with data in the CHR database, the prescription rate (Thrane & Sorensen, 1999) for the target group, i.e., number of animals registered within the age group on herd level, can be calculated. Standardization of usage at farm level relative to number of animals is sensitive to the data quality of the CHR-register. Currently, the Danish authorities are addressing the data quality in the Central Husbandry Register.

Employment of the VetStat data. The information on antimicrobial usage in animals can be employed to support prudent use of antimicrobials on different levels:

- Control and intervention to ensure compliance with developed strategies and regulations on the use of antimicrobials,
- Assisting in the interpretation of resistance surveillance data
- Providing information for research in specific use conditions that govern selection and dissemination of resistant bacteria
- Providing vital information for risk assessment of resistance development at the population level.

Prudent use is further supported by providing direct access for veterinary practitioners and farmers to data concerning their own clients or herds via the Internet. The practitioner and the farmer can compare the standardized usage on the individual farm with the mean usage in similar herds on a regional or national level, thus assisting in pointing out herds with potential management problems. As a tool for optimizing the usage, direct linking with bacterial antimicrobial susceptibility data collected at the Danish Veterinary Institute (isolates from surveillance and clinical specimens) at farm level will be provided.

The authorities are using the data for regulatory control of antimicrobial usage and monitoring of the impact of new legislation. With an unrestricted access to data via the Internet, the authorities may use the system to point out farms with a particular high or low drug, as well as irregularities in regard to the legislation, and identifying the responsible practitioners.

A major objective of the VetStat program is to provide detailed data for research purposes. The veterinary usage of antimicrobial drugs is usually measured in weight of active compound, despite clear limitations as a measure usage and its impact on development on resistance (Chauvin et al., 2001). Taking into account the antibacterial potency, mode of activity, and formulation of the drug provides a more accurate measure of the relative importance of different antimicrobial drugs for generating bacterial resistance. The data enables modeling of associations between usage and resistance, including spatial characteristics and potential seasonal influences and other specific use conditions that may govern selection and dissemination of resistant bacteria.

Since 1996, data on drug usage in Denmark have been used in the interpretation of data from surveillance programs on antimicrobial resistance in the annual DANMAP reports. In the DANMAP 2001 report, Vetstat data were employed for the first time (Anonymous, 2002). The detailed consumption data are expected to give a significant contribution to the interpretation of the data from the surveillance of antimicrobial resistance and in assessing the actual risk to human from the use of antimicrobials in animal production. Linking the usage to susceptibility data may also be performed at herd level to analyze differences between groups of farms e.g. with certain animal husbandry systems, or to support the differentiation between selection of resistance and clonal spread of resistant strains. The VetStat information also enables pharmaco-epidemiologic research programs, analyzing the association between antimicrobial use and morbidity, use of vaccines or other medicines and management factors. Finally, VetStat information might be used in risk based control strategies in the food chain, reducing the spread of resistance genes and resistant bacterial infections from animals to humans.

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Salmonella typhimurium phage types linked with pigs and their association with human infection in England and Wales

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Summary: The most common *Salmonella* isolated from pigs in the United Kingdom is *Salmonella enterica* serovar Typhimurium. Phage typing continues to be a useful method for strain differentiation of this serovar and the predominant phage types in pigs, include definitive phage types (DT) 104, 104b, 193 and provisional phage type (PT) U302. In 2002 the Laboratory of Enteric Pathogens (LEP) reported on 1221 human cases infected with strains of these phage types. Although some of the infections were linked to pig products the majority were due to other sources. However, *S. Typhimurium* DT 208 resistant to tetracyclines (R-type T) or resistant to sulphonamides, tetracyclines and trimethoprim (R-Type SuTTm), PT U310, R-type T and DT 193a, (type first defined in the year 2000), resistant to ampicillin, sulphonamides, tetracyclines and trimethoprim (R-type ASuTTm) appear to be predominantly pig related and were implicated in 160 human infections in 2002, as reported by the LEP.

Keywords: Resistant types, epidemiology, isolated, outbreaks

Introduction: Over two hundred and sixty phage types can be identified using the extended *Salmonella* Typhimurium phage-typing scheme (Anderson et al 1977). This scheme has proved to be invaluable over many years in monitoring and detecting incidents and outbreaks and the emergence and spread of new and old strains of phage typable *S. Typhimurium*. Of the established types DT104, DT104b, DT193 and to a lesser extent provisional phage type U302 (PTU302) continue to be reported from pigs and pig products. However it is the new pig related types of *S. Typhimurium* DT193a and PTU310 that have given recent concern.

Epidemiology: Although the number of human infections of *S. Typhimurium* in England and Wales have fallen in recent years with 1972 human isolates reported by the Laboratory of Enteric Pathogens (LEP) in 2002, *S. Typhimurium* continues to be the second most common serovar after *S. Enteritidis* in England and Wales. The multi-drug resistant strain of DT104 has been the most common phage type of *S. Typhimurium* for well over a decade, since it first appeared in bovines in 1988. In the nineties in addition to strains of bovine origin, *S. Typhimurium* DT104 with multiple resistance was increasingly being isolated from poultry and pigs and to a lesser extent from sheep. However the majority of the recent outbreaks of DT104 have been of poultry origin, particularly turkey. Multi-resistant *S. Typhimurium* DT104b has a similar epidemiology to DT104 but the number of pig isolates have increased particularly in Northern Ireland and Eire (LEP Data). The two most common resistant types found in pigs and pig products are resistance to ampicillin, chloramphenicol, streptomycin, spectinomycin, sulphonamides and tetracyclines, (R-type) ACSSuSpT and with additional resistance to trimethoprim, R-type ACSSuSpTTm.

A well established strain of *S. Typhimurium* is DT193. This type was isolated from pigs in the early nineteen seventies when the type was first identified. Although its major impact was in bovines with its relationship to *S. Typhimurium* DT204 in the late nineteen seventies (Threlfall et al 1978), the strain continues to be isolated from pigs and pig products. The majority of pig isolates are resistant to one or more antibiotics. The dominant resistant types are tetracyclines alone (R-typeT) and multi-resistance to ampicillin, streptomycin, sulphonamides and tetracyclines (R-type ASSuT). Strains of *S. Typhimurium* DT193 have been implicated in a number of human outbreaks where pig products have been vehicles of infection (Maguire et al 1993).

In recent years new phage types of *S. Typhimurium* have been defined by the LEP that are predominantly of porcine origin. Two such examples are DT193a and PTU310. *S. Typhimurium* PTU310 was first described in 1997, resistant to ampicillin, chloramphenicol, streptomycin, sulphonamides, spectinomycin and tetracyclines (R-type ACSSuSpT) but in 2000, 89% of the human isolates were resistant to tetracyclines and 119 of 134 pig isolates typed by the LEP between 1997 and 2000 were resistant to tetracyclines alone (Ward and Threlfall 2001). This trend has continued and in 2001 of 80 human isolates reported by the LEP four were fully sensitive to antimicrobial agents, 74 were resistant to tetracyclines and the remaining two strains had the R-types ACSSuSpT and ASuT. Similarly in 2002 of 68 human isolates, one was fully sensitive, 60 resistant to tetracyclines and seven had different R-types. In the first six months of 2003 there have been twenty six human reports of PTU310, two have the R-type ACSSuSpT and the remainder are resistant to tetracyclines. Twenty of the cases infected with PTU310, R-type T had attended a restaurant carvery where the epidemiological evidence implicated roast pork and stuffing as the vehicle of infection. Of 48 pig isolates of *S. Typhimurium* PTU310 examined between 1st January 2003 and 30th June 2003 four are fully sensitive and 44 have resistance to tetracyclines. Bacterial cell x 24,000

S. Typhimurium DT193a was first defined by the LEP in 2000. This strain is usually resistant to ampicillin, sulphonamides, tetracyclines and trimethoprim (R-type ASuTTm) and is possibly related to DT208 a pig strain that has resistance to tetracyclines alone or sulphonamides, tetracyclines and trimethoprim. In 2002 of 46 human reports of DT193a, 41 had the R-type ASuTTm. Between 1st January and 30th June 2003 the LEP has reported on 80 human isolates of DT193a, R-type ASuTTm. Over 60 of the cases were from the South West region of England and the majority were epidemiologically linked to ham supplied by a butcher in the Bristol area.

The LEP data for 2001 and 2002 on human cases in England and Wales infected with *S. Typhimurium* phage types associated with pigs and pig products is shown in the Table.

Table Salmonella Typhimurium Human England and Wales 2001 – 2002

| PT | 2001 | 2002 |
|-----------------------|------|------|
| 104 | 832 | 748 |
| 104b | 118 | 144 |
| 193 | 194 | 142 |
| 193a | 53 | 50 |
| 208 | 46 | 42 |
| U302 | 77 | 74 |
| U310 | 80 | 68 |
| Remaining phage types | 766 | 704 |
| Total | 2166 | 1972 |

Conclusion: *S. Typhimurium* phage typing together with antimicrobial resistance screening are valuable techniques used in rapidly linking cases of *Salmonella* with food vehicles. Strains of *S. Typhimurium* including new phage types found in pigs, have been implicated in human infections. To control and restrict salmonellosis early detection is essential.

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QUANTIFICATION OF THE SPREAD OF *SALMONELLA* AND THE EFFECT OF 2 FEED ADDITIVES

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Summary: The aim of this experiment was to quantify the spread of *Salmonella* Typhimurium in weaned piglets by calculating R_0 values. In addition, the effect of 2 feed additives, AROMABIOTIC[®] and Shiitake mushroom, on the spread of *Salmonella* Typhimurium was evaluated. Fifty-nine piglets were allocated to 4 groups: AROMABIOTIC[®] (A, n=16), Shiitake mushroom (B, n=16), positive (C, n=16) and negative controls (D, n=11). Half of the piglets of group A, B and C were experimentally inoculated with *Salmonella* Typhimurium, other piglets were used as susceptibles. Based on *Salmonella* isolation in faecal swabs, R_0 values (95% C.I.) were 4.26 (1.40-34.69), 1.83 (0.86-9.38) and 4.29 (1.09-13.71) for group A, B and C, respectively. None of the measured parameters did indicate significant differences between the groups. Mortality rate was high in all 4 groups, what makes it necessary to repeat the experiment.

Introduction: *Salmonella* in swine is mainly transmitted by the faecal-oral route (Fedorka-Cray et al., 1994; 1995), although some authors believe airborne transmission is also possible (Proux et al., 2001). To our knowledge, R_0 values for *Salmonella* have never been calculated before. The aim of this experiment was to calculate reproduction ratios (R_0), defined as the mean number of new infections arising from one typical infectious case introduced in a totally susceptible population, for *Salmonella* in experimentally inoculated weaned piglets. In addition, the effect of 2 feed additives, AROMABIOTIC[®] and Shiitake mushroom (Nutrition Sciences N.V.), on the spread of *Salmonella* was investigated to evaluate whether these additives might be useful to reduce *Salmonella* prevalence.

Materials and methods: Fifty-nine weaned piglets were allocated to 4 groups: group A received AROMABIOTIC[®] (n = 16), group B received Shiitake mushroom (n=16), group C and D were the positive (n = 16) and negative (n = 11) controls, respectively. Piglets were housed with 4 pigs per pen. In each pen of group A, B and C, 2 randomly selected piglets were experimentally inoculated with 1.98×10^9 CFU of a field strain of *Salmonella* Typhimurium. The 2 contact pigs were susceptibles. During the first 2 weeks, the feed for piglets from group A and B was supplemented with 0.3% of AROMABIOTIC and 0.2% of Shiitake mushroom, respectively, according to the recommendations of the manufacturer (Nutrition Sciences N.V.). The next 3 weeks the feed was supplemented with 0.3% AROMABIOTIC and 0.3% of Shiitake mushroom. The active part of AROMABIOTIC[®] is composed of medium chain fatty acids (MCFA), namely caproic acid (C6), caprylic acid (C8) and capric acid (C10) (Dierick et al., 2002). The second additive consists of complete ground Shiitake mushroom (*Lentinus edodes*), a basidiomycete. From day 0 until day 42 post exposure (PE), individual faecal swabs for qualitative *Salmonella* isolation were taken twice a week. After necropsy at day 42 PE, the mesenteric lymph nodes were collected for qualitative

Salmonella isolation using standard procedures. All piglets were blood sampled at day 0, 7, 14, 28 and 42 PE for determining the presence of *Salmonella* antibodies. Starting from day 0 PE, clinical and faeces scores were recorded from every piglet twice a week. In each group the number of dead piglets was recorded to calculate the mortality rate. At the end of the trial, at day 42 PE, all piglets were euthanised. The spread of *Salmonella* in the different groups (experimental unit) was quantified by calculating the basic reproduction ratio (R_0) following the maximum likelihood estimator. Piglets were included in the analysis if at least 3 faecal swabs were available. A pig was considered infected if at least 25% of the faecal swabs were bacteriologically positive, else the pig as considered as not infected.

Results: The R_0 values (95% confidence interval) in group A, B and C were 4.26 (1.40-34.69), 1.83 (0.86-9.38) and 4.29 (1.09-13.71), respectively. The overall R_0 for group A and C was significantly larger than 1, whereas the overall R_0 for group B was not significantly larger than 1. The R_0 values in the 3 different groups were not significantly different from each other. The proportion of lymph node positive animals in group A, B, C and D were 5/13, 5/11, 2/10 and 0/10, respectively ($p = 0.08$). All animals were seronegative at day 7 PE. In all groups, except for group D, a rise in S/P ratio was seen with a peak at day 28 PE. Diarrhea could be observed in all 4 groups during the first 3 weeks of the trial. Besides that, piglets suffered from a *Streptococcus suis* infection, causing arthritis and meningitis. For ethical reasons, all piglets were consequently treated with amoxicillin (Duphamox™) and flunixin meglumine (Finadyne™). Mortality rate in group A, B, C and D was 3/16, 5/16, 6/16 and 1/11, respectively ($p = 0.33$).

Discussion: For the calculation of the R_0 values, an animal was only included if at least 3 faecal swabs were available, what we considered a period long enough to get infected with *Salmonella*. An animal was considered infected if at least 25% of the swabs were positive. This criterion was used to avoid false positive cases due to cross-contamination. Because the entire infectious period of *Salmonella* in pigs is not yet known, the experiment was limited to the nursery period (i.e., 42 days), which differs from the general definition of R_0 (in which R_0 is the average number of secondary infections caused by one typical infectious animal in its entire infectious period). The management and housing conditions of nursery piglets in commercial swine herds were simulated as well as possible, because in the field *Salmonella* infections often occur during the weaning period and feed additives are often used during that period.

During the trial, a high number of piglets became ill and the overall mortality rate was high. A combined *Salmonella* and *Streptococcus suis* infection was diagnosed, what made an overall treatment with amoxicillin necessary. The impact of this treatment on the spread of *Salmonella* is not known but because the *Salmonella* strain used in this experiment was resistant to amoxicillin, this treatment probably may not have influenced results.

Since the R_0 values were above 1, spread of *Salmonella* was continuing in all pens. Because pigs can easily get re-infected due to contact with contaminated faeces, the same situation in the field can be expected.

Conclusions: The results of this experiment indicated that it is possible to quantify the spread of *Salmonella* in pigs by calculating R_0 values. Shiitake mushroom tended to limit the spread of *Salmonella* spp. in weaned piglets, but further experiments are necessary to confirm these results.

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O 72 Implementing a Salmonella Monitoring Programme for Pork in Germany

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Keywords: organizational structures of the swine industry, quality management, central data collection and processing

Summary: The history of the development of a national *Salmonella* monitoring system and its implementation as the "Salmonella Monitoring and Reduction Programme" in the framework of the development of the voluntary German "Quality and Safety System" (= "QS-System") for food production beginning with the pork production chain is explained. Especially the necessary steps that need to be taken to achieve the acceptance for the implementation of an industry-wide programme in a non-integrated pork industry are alluded to.

Introduction: The basic tools, which are necessary for a Salmonella monitoring and reduction programme for a pork production company or chain, for a region, or an entire national pork industry are known and available. The programme can be based on culturing standardized samples from pigs, feed and farm material as still done in the Scandinavian countries Sweden, Norway and Finland (Engvall et al., 1999), or on the semi-quantitative estimation of the *Salmonella* load of herds, i.e. the risk of herds to carry *Salmonella* spec. into the food production chain via live slaughter pigs, by means of standardized random samples of sera or meat juice from pigs for investigating the frequency of antibodies to *Salmonella* spec. Both methods lead to a quite good benchmarking system comparing the potential *Salmonella* loads of market pigs producing herds, which in its turn leads to the possibility to implement targeted measures for reducing the introduction of *Salmonella* spec. into the slaughter house by slaughter pigs on the one hand, and for reducing the cross-contamination of *Salmonella* spec. from salmonella-infected and/or contaminated pigs onto salmonella-free carcasses during the slaughter process.

However, although the principles are well known and well proven in some countries, their implementation is not easy, especially in non-integrated pork industries. The paper describes the lessons learned from the successful implementation of the "Salmonella Monitoring and Reduction Programme" in the framework of the development of the voluntary German "Quality and Safety System" (= "QS-System") in Germany

The developmental stages of the implementation

1. After the start of the "Danish National Salmonella Surveillance and Control Programme" in the early 1990's (Mousing et al., 1997), a joint investigation on:

- a) the prevalence of *Salmonella* spec. in healthy slaughter pigs (cecal content and lymph nodes) and of *Salmonella* antibodies in healthy slaughter pigs (Danish mix-ELISA), and
 - b) the suitability and usability of the Danish mix-ELISA in the German pork industry
- was carried out by several cooperating German institutions (the Fed. Institute of Consumer Health Protection and Veterinary Medicine in Berlin, the Department of Epidemiology of the Federal Institute of Viral Animal Diseases in Wusterhausen, and the Field Station for Epidemiology of the School of Veterinary Medicine of Hannover in Bakum) under the auspices of the federal Ministries of Health and Agriculture (Kaesbohrer et al., 1998).

2. The result of this joint study was that the prevalence of *Salmonella* spec. and the range of serovars found by culturing cecal contents and ileocecal lymph nodes as well as the prevalence of salmonella antibodies detected in 1995 to 1996 were comparable to those that were published by the Danish

Association of Pork Producers and Slaughterhouses and by researchers that had carried out similar explorative studies in other countries such as The Netherlands, Austria etc. It was also concluded that the Danish mix-ELISA was suitable and usable in Germany for a semi-quantitative categorization of market pig herds into three risk levels: low risk, medium risk and high risk based on the experiences from Denmark. Consequently, the Ministry of Agriculture recommended the implementation of a Salmonella Monitoring Programme addressing the farming community and the slaughter and meat processing industry.

3. Several attempts were undertaken to start such a programme on a voluntary basis. However, the effect of the wide range of the organizational structure in the German pork industry and the effect of the competition of the many slaughter plants (> 260 in Germany) for slaughter pigs was underestimated. It is understandable that no slaughter plant wanted to be the first to tell farmers that they are to do something to reduce their *Salmonella* load, since the more or less unprepared pork producers interpreted the testing for *Salmonella* spec. as tool for downgrading their products and started to sell their pigs rather to a slaughter plant that had not (yet) started to test for salmonella antibodies. In other words, a salmonella surveillance programme as marketing tool is easily understood as improvement of the competitiveness of the final pork product in an integrated system exporting pork such as that of Denmark, which needs tools for market leadership in the international market. The same programme without a mechanism for guaranteeing that all slaughter plants start the programme at the same point in time, becomes a "threat" to the farming community in a non-exporting and non-integrated swine production structure such as that of Germany, and even to the forerunners in the slaughter industry as well, since the "frightened" farmers will quit their supply and change their market partner.

4. As consequence, the Ministry of Agriculture understood the necessity of a legal tool for forcing the implementation of such a programme on all participants in the pork industry as a governmental "wish" to gradually reduce the introduction of *Salmonella* spec. into the pork chain.

However, there are several legal difficulties to overcome if there is to be on the one hand the duty of all farmers and slaughterhouses to participate, and on the other hand not to interfere with the principles of the free market system (for instance how to split the costs between the farmers and the slaughterhouse and many other questions). The issue of the planned governmental directive was several times postponed on legal grounds.

5. Meanwhile, the political "climate" towards food production had changed due to the BSE discussions throughout Europe. The Ministry of Consumer Protection and Agriculture, "run" now by Green Party, had started to promote organic food and the declaration of production methods. This led to the need for the "conventional" agricultural production community to develop a quality assurance programme assuring the consumer and the market that the production procedures follow certain quality requirements. The "QS-System" (= a food **Q**uality and **S**afety Management System for guaranteeing the compliance with basic quality criteria throughout the food production chain) was designed through a concerted action between the feed industry, the farmers association, the slaughter industry, the processing industry and the retailers of Germany (Anonymous, 2003). One of the major QS-system modules is the implementation of the "Salmonella Monitoring and Reduction Programme". The participation in the QS system is voluntary, however, for those that decide to participate in QS, the compliance with the salmonella programme is mandatory (more than 1/3 of the German pork producers are participating, which is more than 2/3 of the German pork production).

6. The programme has officially started for all QS participants on April 1, 2003. The guidelines for every partner in the programme (the farmers, the slaughter plants, the laboratories conducting the ELISA test, the central data base, third party auditors and consultants such as veterinary practitioners are published at the QS-system's home page (Anonymous, 2003).

The programme is based on a continuous sampling of meat juice from 60 pigs per herd and year, after the first 60 samples (under 400 pigs per herd there had to be smaller random samples per year, but categorizing starts only after 60 samples). The meat juice samples are tested for *Salmonella*

antibodies with three ELISA tests with the cut-off value of 400D% - the laboratories and the tests are approved by the QS-system (Blaha et al., 2003). The participating herds are categorized into low risk (= < 20% of the samples are serologically positive), medium risk (= > 20% but < 40% of the samples are serologically positive), and high risk (= > 40% of the samples are serologically positive). Starting in April 2004, high risk herds will be slaughtered separately and they will have to implement a farm specific *Salmonella* reduction plan according to the identified specific *Salmonella* sources.

Lessons learned

To achieve the goal that in countries with a highly diversified and non-integrated pork industry a national *Salmonella* monitoring system is accepted by all players in the pork chain and results in a control and reduction programme that the market and the public understand as a doubtless improvement of the national pork production, the following measures need to be taken:

- 1) make sure that all parts of the pork chain have understood the programme's intention before any activity for its implementation (assure the farmers that it will not be misused against them, and prevent processors to misuse it in the competition against each other),
- 2) make sure that everybody understands that a central data base is inevitable for the success of the programme (assure everybody that the data base is accessible to only authorized persons or parties),
- 3) make sure that farmers that are categorized as "high risk" farms get help instead of discrimination (many a well managed high health, high biosecurity, high hygiene farms is initially categorized as *Salmonella* high risk farm!).

Communication for consensus amongst the players in the pork chain is the key of success.

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SLATTED PEN FLOORS REDUCE *SALMONELLA* IN MARKET SWINE HELD IN ABATTOIRS

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Summary: The objective of this study was to directly compare *Salmonella* isolation rates from pig slaughtered after 4 hours holding on slatted or solid concrete floors. Seven truckloads (replicates) of market swine (120 kg) from a fully integrated farrow-to-slaughter operation were studied. At unloading, groups of pigs (15-30) were sorted to 1) no-hold (20-45 minutes waiting), 2) pens with solid concrete floors (4 hours), 3) slatted concrete floors (4 hours). Postmortem samples were cultured for *Salmonella*. Those pigs held in pens with slatted floors (63.6%) had significantly ($P < 0.05$) less *Salmonella* in their ceca than those held on solid floors (72.7%).

Keywords: food safety, zoonosis, abattoir, lairage

Introduction: In the US, market swine spend two to six hours resting in holding pens before slaughter. This time is necessary for ante mortem inspection and improvement of meat quality. However, research is beginning to suggest that these holding pens may be a significant risk for *Salmonella* infection and a potential critical control point (Hurd *et al.*, 2001; Hurd *et al.*, 2002; Rostagno *et al.*, 2003). The objective of this study was to directly compare *Salmonella* isolation rates from pigs slaughtered after 4 hours holding on slatted or solid concrete floors.

Materials and Methods: Seven truckloads (replicates) of market swine (120 kg) from a fully integrated farrow-to-slaughter operation were studied from June 2003 to February 2003. For each load, all pigs ($n=170$) originated from the same building. They were transported less than one hour in clean and disinfected trailers. All groups consisted of pigs marketed at close-out (emptying) of the building. At the time of unloading, small groups of pigs (15-30) were sorted to 1) no-hold (20-45 minutes waiting), 2) pens with solid concrete floors (solid), 3) slatted concrete floors (slatted). The latter two groups were held for approximately four hours. At harvest, samples were collected from 30 animals from each of the three groups. Following stunning and evisceration, cecal contents, feces, and ileocecal lymph nodes (ICLN) were collected from the viscera line.

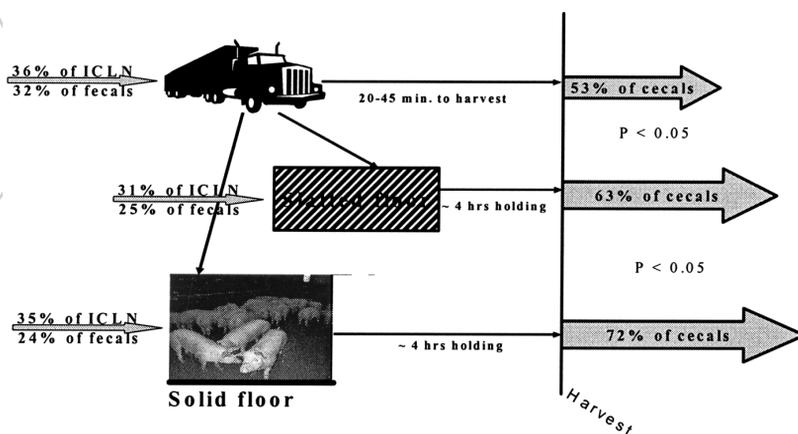
For *Salmonella* isolation, samples (macerated ICLN, 10g feces, 10ml cecal contents) were added to both buffered peptone water and Tetrathionate (90 ml) which were incubated for 24 h at 37 °C. Each was then subcultured 1:100 in Rappaport-Vassiliadis broth + novobiocin (20mg/L), incubated for 24 h at 42 °C and then transferred 1:100 to Rappaport-Vassiliadis broth and incubated 24 h at 42 °C. Samples from the BPW pre-enrichment were screened using the Assurance gold EIA *Salmonella* ELISA kit (BIOCONTROL, Bellevue, WA). ELISA positive BPW samples and all samples from the Tet enrichment were then streaked onto XLT4 and modified brilliant green agar (MBG) and incubated for 24 h at 37 °C. Suspect colonies were then confirmed by isolation of typical colonies on Rambach agar (24 h at 37 °C) and serotyping by USDA National Veterinary Services Laboratory.

Results and Discussion: A total of 630 pigs were evaluated. Those pigs held in pens with slatted floors (63.6%) had significantly ($P < 0.05$) less *Salmonella* in their ceca than those held on solid floors (72.7%). There was no difference in the prevalence from ICLN or fecal samples between the solid and slatted groups (Figure 1). For all three groups, the overall proportion of positive ceca (62.8%) was higher than the ICLN (33.8%) or the feces (26.85). Based on previous studies we suspect that ICLN

prevalence largely reflects the on-farm prevalence (Hurd, *et al.*, 2003). For this reason, the ICLN prevalence is represented as an input on the left side of Figure 1. The cecal prevalence more likely represents the recent exposures occurring in the last four hours of life (holding pen) and is represented as an outcome of the holding process.

This study suggests that dry slatted floors may provide some benefit in reducing preslaughter *Salmonella* exposure. The slight dose-response observed with increasing cecal prevalence from no-hold to solid floors supports this hypothesis of causality. The observation of higher cecal prevalence in the no-hold pigs due to recent exposure is supported by our other work showing cecal contamination in as little as 15 minutes (Griffith, *et al.* 2003). The effect of the holding pen exposure is partially obscured by the relatively high on-farm prevalence, as measured in the ICLN. Further analysis, involving the comparison of serovars (and PFGE fingerprints) found between groups will better delineate the role of holding pens and possibilities for intervention.

Figure 1. *Salmonella enterica* recovery rates from market swine at slaughter after three types of lairage, 1) 20-45 minutes, 2) 4 hours on solid concrete floor, 3) 4 hours on slatted concrete floor.



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Reduction of *Campylobacter* and *Salmonella* in pigs treated with A select nitrocompound

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Summary: The aim of this study was to test the effectiveness of administering a select nitrocompound (S-NO) on reducing naturally colonized *Campylobacter* and experimentally infected *Salmonella* in the weaned pig gut. Pigs were divided into four groups; control (0 g S-NO/pig), 1X (0.2 g S-NO/pig), 5X (1 g S-NO/pig), and 10X (2 g S-NO/pig). Treatments were administered via oral gavage 24 h before sacrifice. Mean \pm SD populations (\log_{10} cfu/g) of *Campylobacter* in the cecum were reduced ($P < 0.05$) in pigs receiving the 10X dose when compared with untreated controls (1.64 ± 1.30 vs 5.31 ± 0.58 , respectively). *Campylobacter* concentrations in rectal contents from pigs administered the 5X dose were reduced ($P < 0.05$) compared to control (2.65 ± 2.86 vs 5.90 ± 0.94 , respectively). Rectal *Salmonella* concentrations were reduced ($P < 0.05$) in all of the S-NO-treated groups. Adverse effects of S-NO on pig health were not observed. These results demonstrate that S-NO may have potential as an intervention to reduce pig colonization by *Campylobacter* and *Salmonella*.

Keywords: antimicrobial, pre-harvest, food safety, intervention, swine.

Introduction: *Campylobacter* and *Salmonella* spp. continue to cause human infections and are implicated as two of the most leading etiological agents of foodborne diseases. Pigs are known to be a major natural reservoir for these pathogens (Weijters et al., 1999; Duijkeren et al., 2002). Some nitroalkanes have been reported as alternative electron acceptors by *Denitrobacterium detoxificans* (Anderson et al., 2000). Hence, these compounds have an antimicrobial activity against *Campylobacter jejuni* (unpublished) and are also effective in inhibiting ruminal methanogenesis *in vitro* (Anderson et al., 2003). The aim of this study was to determine the bactericidal activity of one of these nitrocompounds, S-NO against *Campylobacter* and *Salmonella* in the cecum and rectum of weaned pigs. We also determined the concentrations of total culturable anaerobes, total coliforms as well as generic *Escherichia coli*.

Materials and Methods: An experiment was conducted using weaned pigs (21-28 d-old) obtained from a local producer and housed in concrete floor pens. Piglets were provided ad libitum access to an unmedicated corn-soybean meal and whey starter diet during entire experimental period. Upon arrival, rectal swabs were collected from each piglet and cultured for the presence of *Salmonella* and *Campylobacter*. Piglets were challenged via oral gavage with 5 ml of a novobiocin and nalidixic-resistant *S. Typhimurium* that had been grown overnight in tryptic soy broth. The concentrations of the challenge dose of *S. Typhimurium* were approximately 2.0×10^9 CFU/ml. Pigs were divided into four groups with unbalanced design; control (0 g S-NO /pig; n = 10), 1 X (0.2 g S-NO/pig; n = 10), 5 X (1 g S-NO/pig; n = 6), and 10 X (2 g S-NO/pig; n = 3). Treatments (S-NO) were administered via oral gavage 24 h before sacrifice. Cecal and rectal contents (1 g) were serially diluted (1:10) in phosphate buffer (PB; pH 6.5) spread plated onto brilliant green agar (BGA) supplemented with $25 \mu\text{g}$ novobiocin ml^{-1} only (N) or with novobiocin and $20 \mu\text{g}$ nalidixic acid ml^{-1} (NN), and to Campy-Cephex agar for enumerating *S. Typhimurium*, *Salmonella*, and *Campylobacter*, respectively. *Escherichia coli* and total coliforms were enumerated using *E. coli*/coliform Petrifilm plates. All plates were incubated at 37°C for 24 h except Campy-Cephex plates which were incubated in a microaerobic environment (5% O_2 , 10% CO_2 , 85% N_2) at 42°C for 48 hours. For qualitative enrichment, cecal and rectal contents were enriched in tetrathionate broth for 24 h and then streaked on BGA for *Salmonella*. For *Campylobacter*, contents were enriched in bolton broth at 37°C for 4 h and then 42°C for 44 h. Suspected colonies on BGA and

Campy-Cephex plates showing typical salmonellae and *Campylobacter* morphology were picked and confirmed biochemically and serologically. Total culturable anaerobes were determined via direct plating on anoxic brucellar agar under CO₂ (5 %). The concentrations of volatile fatty acid (VFA; acetic, butyric, propionic, isobutyric, valeric, and isovaleric) were determined by gas chromatography (Hinton, et al., 1990). The bacterial populations recovered on plates were transformed to log₁₀ CFU for statistical analysis. Qualitative enrichments resulting no growth on BGA plate were assigned a value of 0.1 log₁₀ CFU/g and enrichment yielding *Salmonella* positive plates were assigned a value of 9 CFU/g. Data were analyzed using the general linear model of Statistical Analysis Systems (SAS, 1999).

Results and Discussion: Although we did not observe any biologically adverse effects after perorally administering S-NO, there were a few piglets showing diarrhea among all groups including untreated control group but this was probably due to the high dose of *Salmonella* administration. Table 1 shows the effect of S-NO on naturally occurring *Campylobacter* populations in the cecum and rectum. More than 3 log unit reductions of *Campylobacter* populations occurred ($P < 0.05$) in cecal and rectal contents from piglets treated with 10 X and 5 X doses of S-NO, respectively. The resurgent increase of *Campylobacter* populations in rectum at 10 X dose-treated group is unclear but may suggest that *Campylobacter* recovered from this group was relatively more resistant to the S-NO compound than those isolates from the other group. Alternatively, environmental factors in the gut such as pH, redox potential or other unknown factors may have diminished the bactericidal activity of S-NO or have perturbed its pharmacokinetic properties in situ. Preliminary *in vitro* results have shown an enhanced bactericidal activity of S-NO against *C. jejuni* under slightly alkaline conditions (pH 8.0) (data not shown). The wildtype *Salmonella* population was reduced ($P < 0.05$) upon all S-NO treated groups in rectum (Fig 1) while we observed no effect on *S. Typhimurium* concentrations (data not shown). This latter result is probably due to an insufficient challenge. In contrast, results from recovered populations of total coliform, *E. coli* and total culturable anaerobes reveal that these bacteria were not affected ($P < 0.05$) by this compound (data not shown). In addition, analysis of acetate, propionate, and butyrate revealed no S-NO effect ($P < 0.05$) on concentrations of these volatile fatty acids in the cecum and rectum of treated pigs (data not shown). These findings suggest that the S-NO treatment did not influence the fermentation profiles within the pig gut. More studies including optimization of concentrations for effective doses, determining the spectrum of activity of this compound, and elucidation of mechanism(s) of action are in progress in our laboratory.

Conclusion: The scope of this study shows that S-NO may have potential applicability as an intervention to reduce pig colonization by *Campylobacter* and *Salmonella*.

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Table 1. Effect of select nitrocompound (S-NO) treatment on cecal and rectal *Campylobacter* populations following natural exposure in weaned pigs.

| Mean log ₁₀ <i>Campylobacter</i> spp. CFU/g of cecal and rectal content ^a (standard deviation) ^a | | |
|---|---------------|---------------|
| Treatment ^b | Cecal | Rectal |
| Control | 5.31 (0.58) A | 5.90 (0.94) A |
| 1 X S-NO | 4.95 (1.02) A | 4.68 (1.71) A |
| 5 X S-NO | 5.30 (0.43) A | 2.65 (2.86) B |
| 10 X S-NO | 1.64 (1.30) B | 5.65 (0.85) A |

^a Weaned piglets (21 to 28 days of age) were used in this experiment; n = 10 for all treatment groups at each cecal and rectal concentration except 5 X (n = 6) and 10 X (n = 3). Means within columns with different letters differ (P < 0.05). There was a significant (P < 0.05) interaction between treatment and sampling location (data not shown)

^b Treatments, 0 (control), 0.2 (1 X), 1 (5 X) and 2 g (10 X) S-NO per pig were administered via oral gavage 16 h post challenge. Cecal and rectal contents were collected 24 h afterwards.

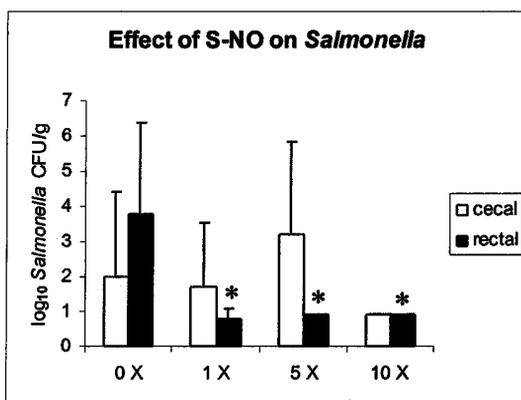
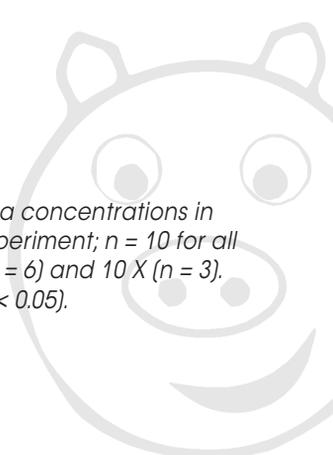


Fig. 1. The effect of select nitrocompound (S-NO) on wildtype *Salmonella* concentrations in weaned pigs. Weaned piglets (21 to 28 days of age) were used in this experiment; n = 10 for all treatment groups at each cecal and rectal concentration except 5X (n = 6) and 10 X (n = 3). Asterisks indicate means differ from 0 X control means (P < 0.05).



CREATING AN INTEGRATED PORK SAFETY AND QUALITY SYSTEM IN GREECE

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Summary: The study for establishing an integrated safety and quality certified system for pork, in Greece, is reported. To this end, the Hellenic Organisation for Certification and Supervision of Agricultural Products (AGROCERT) created standards for feed production, pig farming, pig slaughtering, meat cutting and pork retail selling points. Certification of the various stakeholders of the pork industry, according to the above standards, by AGROCERT, is a prerequisite for entering to the system. The system is to be operated by an independent agency (DELFA), to which production – processing – distributing units are participating, regardless of their specific activity, in the chain from production to the retail selling points. The agency is responsible for maintaining the basic characteristics for the credibility and transparency of the system, in order that certified pork will be distinguished in the market by means of a specific trade mark, in combination with the trade mark of AGROCERT.

Keywords: certification, trade marks, "stable to table"; trace ability, quality control

Introduction: One of the current attitudes of the consumer, in developing markets, is his/hers mistrust to food producers – processors – distributors, as well as to regulatory and food inspection authorities. The reaction of the stakeholders is to establish integrated, transparent and credible food safety and quality certified systems. In the EU, this is in line with the White Paper on Food safety (2000) and the Regulation 178/2002, which are generally supporting, among other things, the concept of integrated approach to food safety.

Within the above framework the stakeholders of the pork industry in Greece commissioned a study for establishing a relevant system for pork in Greece, which is reported below.

THE PREREQUISITES

In order to claim a certain level of safety and quality, as well as in fulfillment of "other legitimate factors", to which the consumer is sensitive, related to sustainability and animal and environmental protection, standards for the different links in the chain of pork production – processing – distribution were established, under the auspices of AGROCERT. These standards are specifications appearing under the general title of "Management System for Pig Meat Quality Assurance". They are the following:

- o AGRO 3-1. Part 1: Requirements for the production of pig feedstuffs
- o AGRO 3-2. Part 2: Farming requirements for pig fattening
- o AGRO 3-3. Part 3: Requirements for pigs slaughter
- o AGRO3-4. Part 4: Requirements for cutting, deboning, processing and packing pig meat
- o AGRO 3-5. Part 5: Requirements for the retailers of pig meat.

At Annexes, in AGRO 3-3, the specifications for transportation of live animals and carcasses are, also, appearing.

THE SYSTEM

Certified implementation of the standards is a prerequisite for entering to the system. Notwithstanding the significance of the above standards, for achieving an assured safety and quality system, linking these standards together in an integrated way is an apparent necessity. Such a linking, serves the

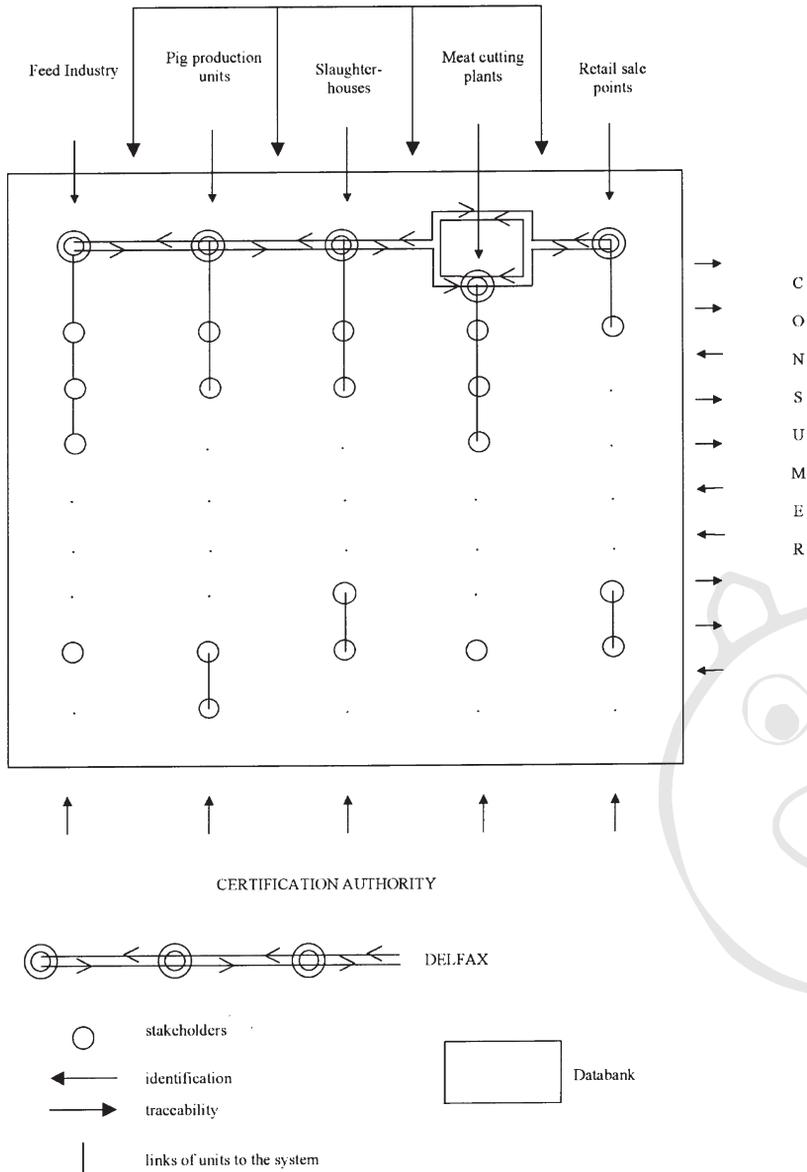
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contemporary concept for assuring food hygiene and quality, which is suggesting an holistic approach "from stable to table".

To support the aforementioned requirements an independent agency, under the name DELFAX*, was created. It will assume the responsibility for preserving the basic characteristics for the credibility and transparency of the whole system, which are:

*DELFAF (Δέλαφαξ) in the Homeric dialect means piglet

Figure 1. Summary of the integrated safety and quality DELFAX system for pork



- o Identification and feedback traceability of a meat cut, ideally, at the level of final selling point
- o Functioning of an electronic data base with unalterable and accessible to the consumer data

Additionally the same agency will be responsible for:

- o Developing and supervising the application, by its members, the regulations governing the internal functioning of the system
- o Training of its members for the in practice application of the system
- o The internal auditing of the system
- o Linking together, in an integrated way all participating units in the system.

In Fig. 1 the whole system is summarily illustrated.

Conclusion: Under these conditions the distribution of certified pork in the market, supported with accessibility of the consumer to all information stored in the data bank, operating by the agency functioning and supervising the system, will be recognized through its special trade mark in connection with the trade mark of AGROCERT. Further it is hoped that the product will enjoy premium prices.

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Commission of the European Communities, 2000. White Paper on Food Safety Brussels, January 2000.

EU regulation 178/2002 of the European Parliament and the Council, of 28 January 2002, laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety.

O 76 Reduction of *Salmonella choleraesuis* contamination in pork carcasses by vaccination

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Summary: *Salmonellosis* is a common clinical and subclinical infection of pigs. The species adapted serovar *S choleraesuis* predominantly produces a septicemic disease in swine. Disease in other species, including humans, is rare compared to enteric type infections from non host adapted serovars such as *S typhimurium*. However, when host adapted serovars infect alternate species, disease can be severe. Vaccination with an avirulent live *S choleraesuis* vaccine, Enterisol® SC-54, significantly reduces prevalence and quantity of infection in pigs. Additionally, those pigs that remain culture positive have two logs or more reduction in the quantity of *S choleraesuis* present. Vaccination of pigs as young as one day of age is successful. Where these infections are of concern in the human population, vaccination of pigs may provide dramatic reductions of the organism swine, reducing risk in food items of swine origin.

Keywords: prevalence, vaccination, Enterisol® SC-54, control

Introduction: *Salmonella* infections in pork have been linked to outbreaks of food born disease in humans. Those practices that reduce the level of contamination or carriage of *Salmonella* in carcasses that arrive at abattoirs should assist in reducing contamination of post-harvest pork products. In some cases, *Salmonella choleraesuis*, a host adapted *Salmonella*, may play an important role in human disease. While typically a rare infection in humans, some countries report significant problems with *S choleraesuis* infections in humans (Chui et al., 2002). In some areas, the prevalence of *S*

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choleraesuis may reach 5% or more of all *Salmonellae* isolated from human samples (Chui et al., 2002). When these isolates become resistant to primary treatment antibiotics, other methods for preventing exposure to people from the resident source, swine, may increase in importance. Challenge control studies were performed to gain USDA licensure of a live, avirulent *S choleraesuis* vaccine, Enterisol® SC-54 (Boehringer Ingelheim Animal Health GmbH). This vaccine was developed by serial passage through neutrophils. One of the mechanisms of attenuation was natural loss of the virulence plasmid, *vpl*, during passage (Roof et al., 1992). The vaccine is fully licensed as safe and efficacious by the USDA and numerous countries throughout the world. This paper summarizes data on reducing the prevalence of *S choleraesuis* by vaccination.

Materials and Methods: Pigs for challenge studies were selected from herds free of clinical signs of salmonellosis in the nursery. For each study, pigs were weighed and blocked by sex and weight into strict control (non vaccinated, non challenged), challenge control and vaccinated/challenged. Group sizes ranged from 10 to 20 pigs for each study group. Vaccine was administered either intranasally or orally, via drinking water, to mimic the natural route of exposure. Animals were housed in separate rooms prior to and during challenge to reduce exposure between groups. The interval from vaccination to challenge ranged from 14 days to 140 days. Pigs were monitored for 14 days following challenge for clinical signs and weight gain. All surviving animals were humanely euthanized at the end of the challenge period. Studies were performed both internally, and by independent laboratories (Baum, 1997).

Results: Data from studies comparing Enterisol® SC-54 to non vaccinated control pigs challenged with *S choleraesuis*. Various tissues were examined for presence (+/-) and quantity (log cfu/gm tissue) of the challenge organism. A biochemical marker was used to differentiate the vaccine from challenge strains. When pigs were vaccinated at three weeks of age or older, either intranasally or orally, via drinking water, pigs were clinically protected from challenge for at least 20 weeks following vaccination. Additionally, both the prevalence and quantity of *S choleraesuis* in internal organs were significantly reduced. Reduction of 1 log or more, and 50% lower rate of organ colonization was noted, all significant at $p < 0.05$.

An additional efficacy study was performed in order to obtain a license to vaccinated pigs at one day of age. Cohort pigs were vaccinated intranasally at one day of age, three weeks of age, or left as strict or challenge control pigs. Challenge with 10^{10} virulent *S choleraesuis* occurred at 34 days of age. Pigs were monitored for 14 days following challenge.

As compared to both control and 21 day vaccinated pigs, the group of pigs vaccinated at one day of age had lower culture prevalence and cfu/gm of tissue of the challenge strain (Table one). Daily gain, temperature post challenge and mortality were nominally improved for one day vs. 21 day vaccination. All vaccinated pigs were significantly improved over control pigs. Organ protection from colonization was significantly improved for one day vaccinated pigs, as compared to both controls and 21 day old vaccinates, which performed consistently with prior studies.

Table 1. Summary of Clinical and Organ Colonization on *Salmonella choleraesuis* challenge

| | Challenge Controls | Day One | Day 21 |
|----------------------------------|--------------------|--------------------|--------------------|
| Mean Temp °C | 41.15 ^a | 39.94 ^b | 40.33 ^b |
| ADG (gm/day) | 254 ^a | 636 ^b | 617 ^b |
| 14 Day mortality | 70% ^a | 0% ^b | 10% ^b |
| % animals positive | 100% ^a | 10% ^b | 50% ^c |
| % organs positive | 73% ^a | 1.4% ^b | 10% ^c |
| Mean cfu/gm (log ¹⁰) | 3.45 ^a | 0.242 ^b | 0.689 ^c |

Means with different superscripts are significantly different ($p < 0.05$)

Discussion: Immunization with an avirulent live *S choleraesuis* vaccine has demonstrated consistent clinical and bacteriologic control of *S choleraesuis* infections in swine. Clinical signs, including febrile response, diarrhea and mortality, along with growth rate, are significantly improved ($p < 0.05$). Pigs from one day of age and older may be safely vaccinated intranasally, or orally, via drinking water, against disease associated with *S choleraesuis*, and to reduce the culture prevalence and shedding of other serovars of *Salmonellae* (Nolan et al., 2000). Additionally, levels of the organism are effectively reduced in vaccinated, challenged pigs. Fecal shedding of *S choleraesuis* is significantly lower in vaccinated challenged pigs as compared to challenged control pigs. Organ culture prevalence is likewise dramatically lowered in vaccinated pigs. Vaccination at one day of age is at least as efficacious, and may offer even greater advantage over non vaccinated controls.

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Reduction of salmonella contamination in pork carcasses by vaccination

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Summary: Two field studies and one laboratory challenge were performed to evaluate of vaccination with a live, avirulent *Salmonella choleraesuis* vaccine (Enterisol® SC-54) to provide cross protection, reduce the level of internal culture and fecal shedding of multiple serovars of *Salmonella*. Barns of grow-finish pigs were vaccinated orally, via drinking water, or left as matched controls in the field studies. Ileocecal lymph nodes and spiral colon fecal material were collected at the abattoir from field studies. Three week old pigs were vaccinated intranasally and challenged at five weeks of age in the laboratory study. Internal organ culture and fecal shedding were measured two weeks following challenge with *S typhimurium*. In all three studies, vaccinated pigs had significantly lower culture prevalence of non *S choleraesuis* serovars ($p < 0.05$), and reduced fecal shedding in following laboratory challenge ($p < 0.05$). A nominal ($p = 0.07$) trend to improved growth rate following laboratory challenge with *S typhimurium* challenge was also detected.

Keywords: prevalence, shedding, food safety, Enterisol® SC-54, performance

Introduction: *Salmonella* infections in pork have been linked to outbreaks of food born disease in humans, attracting attention to those strategies that may help reduce *Salmonella* in pork (Letellier et al., 2001). Those practices that reduce the level of contamination or carriage of *Salmonella* in carcasses that arrive at abattoirs should assist in reducing *Salmonella* contamination of post-harvest pork products. Enterisol® SC-54 (Boehringer Ingelheim Animal Health GmbH), an avirulent live *Salmonella*

choleraesuis vaccine, has been utilized successfully to control clinical disease associated with *Salmonella choleraesuis* in pigs (Roof et al., 1992). The purpose of these studies was to demonstrate reduction of *Salmonella* shedding and culture prevalence in carcasses (Baum, 1997) and reduction of sero-prevalence at slaughter (Nolan et al., 2000) in commercial production systems, and following a laboratory challenge. These studies included reduction of *Salmonella* contamination of carcasses at harvest in two conventional production systems, and a reduction in culture and shedding *S typhimurium* in a laboratory challenge.

Materials and Methods: In two large field studies (>50,000 pigs), grow-finish pigs from two production systems were randomly assigned at placement to finishing into either vaccinated or control groups (barn = Exp. Unit). Control and vaccinated barns were matched by animal source, feed source, field management, barn type and season. In most cases control barns were housed at the same location as vaccinated pigs. Vaccine was administered orally, via drinking water, at placement to grow-finish. Facilities were managed in an all in/all out fashion. Total slat, confined finishing barns were used for all groups in study one. Study two utilized partially slatted finishing barns. Other variables that might affect *Salmonella* contamination, e.g. feed type and source, pig source, pre-harvest handling, etc, were consistent between each group (Dahl et al., 1996). Each treatment consisted of 12 or more groups of animals in each study.

A random sample of 35 pigs from each group were selected from either the middle (study one) or last marketing group (study two), and transported by clean, disinfected livestock trailers to the abattoir. Pigs were held for a maximum of three hours prior to harvest. Power calculations for selecting sample size were based on alpha <0.05 and beta >0.8 statistical criteria.

Pig identity was established, and viscera sets (lung, liver, intestines) were individually bagged in clean plastic bags. The viscera were removed to a separate clean room at the facility for collection of ileocecal lymph nodes (study one and two) and spiral colon fecal content (study two only). Additionally, blood and diaphragmatic samples were collected from a random subset of pigs. Culture processes and organ selection followed previously described methods (Hurd et al., 2001). Field collection and laboratory personnel were blinded to source (vaccinated or control) during culture, serotyping and external identification of serovar. The Danish Mix ELISA test was utilized to determine the correlation of group culture status vs. serologic status.

In the laboratory challenge, three week old pigs were obtained from a farm with no history of clinical Salmonellosis in nursery age animals. Pigs were blocked by weight and sex, and randomly assigned to vaccinated (n=20), strict control (10) or challenge control (10) groups. Vaccinated pigs received one dose of Enterisol® SC-54 intranasally, while challenge controls received diluent placebo. Pigs were maintained for two weeks in isolated rooms on wire flooring prior to oral challenge with virulent *S typhimurium* to allow for development of immunity. Fecal culture was performed in all groups both prior to and during the challenge study period.

Vaccinated and challenge control pigs were challenged orally with >10⁸ virulent *S typhimurium*. Pigs were evaluated for clinical signs, weight gain and fecal shedding for a period of two weeks following challenge. At the end of the two week period, animals were humanely euthanized and internal organs (lung, liver, spleen, lymph node) and fecal samples collected for bacteriologic culture. Methods utilized have been previously described (Hurd et al., 2001).

Results: Vaccinated pigs demonstrated a statistically significant reduction in the percent of carcasses culture positive for *Salmonella* at slaughter in both field studies. In study one, vaccinated pigs had 12% of ileocecal lymph nodes culture positive for *Salmonella* species as compared to 24% of non vaccinated pigs (p<0.05). On serologic examination, 14% of vaccinated pigs were seropositive on

the Mix-ELISA, significantly less than the 26% of seropositive control pigs ($p < 0.05$). Individual serum samples were considered positive on Mix-ELISA at OD % 40 or above.

In study two, vaccinated groups of pigs averaged 7.4% culture positive in ileocecal lymph nodes, as compared to 26.8% in non vaccinated controls, again a significant reduction ($p = 0.03$). Prevalence in non-vaccinated control pigs was similar to other surveys in both studies (Hurd, 2002). Reduction was significant for all *Salmonella* species, and non *S choleraesuis* species as a group. *S typhimurium* and *S derby* were the most commonly isolated organisms from the ileocecal lymph nodes. Vaccinated groups averaged 10.2% seropositive, while non vaccinated control groups had a mean of 24% seropositive. Due to a wide range of seroprevalence in controls ($sd = 31\%$), the difference between groups was not significant at the sample size utilized ($p > 0.1$).

A trend toward reduction of *Salmonella* prevalence in spiral colon fecal samples was also noted in study two ($p = 0.1$). Thirty-two percent of non-vaccinated pigs had *Salmonella* detected in fecal samples, as compared to 12.9% in vaccinated pigs. The relatively small number of study groups (only 10 groups of each treatment were collected) compared to the high variability in control group fecal culture prevalence ($sd = 37.5\%$) suggests insufficient power in the fecal sample portion of the study. A sample size of 43 groups would be required for alpha < 0.05 with the same means, and the same standard deviation in the control pigs. *S typhimurium* and *S derby* were the most commonly isolated organisms from spiral colon fecal samples.

The correlation r^2 of group culture status vs. serologic status was 0.67. Culture positive was defined as one or more ileocecal lymph nodes culture positive. Seropositive groups were defined as one or more serum samples positive on Mix-ELISA (OD % 40 or above). One group of pigs was 57% culture positive (13 positive for *S derby*, 2 for *S anatum* and 1 *S typhimurium*) at slaughter but was seronegative. Pen fecal samples collected prior to harvest of this group contained *S derby* and *S typhimurium*, suggesting a late finishing exposure to *S derby*. This demonstrates the limits of categorization on the basis serology using a point in time or intermittent basis, as compared to utilizing serology as part of an ongoing sampling of a population of animals.

In the laboratory challenge study, at no point in time did strict (non challenged) controls have detectable *Salmonella* in fecal or organ culture, indicating effect control of unintentional exposure. Only three of twenty vaccinated pigs had detectable *S typhimurium* in organs or feces at post mortem 14 days following challenge. In contrast, all 10 control pigs were positive. Thirteen of 20 vaccinated pigs shed *S typhimurium* for one or more days during the study period, while seven vaccinated pigs did not have detectable shedding. All ten control pigs shed *S typhimurium* for at least one day during the study period. Both measures showed significant ($p < 0.05$) reduction in *S typhimurium* in organs or feces in pigs receiving a live avirulent *S choleraesuis* vaccine. These findings are consistent with those of Baum (1997) that demonstrated an association of *Salmonella* exposure with reduced performance.

Vaccinated pigs did not seroconvert on the Danish Mix-ELISA. Subsequent examination of serum samples using a commercial *Salmonella* LPS ELISA (Idexx Laboratories, Westbrook, Maine) has shown similar lack of seroconversion in pigs vaccinated with Enterisol® SC-54. One possible explanation for this phenomenon is the lack of the *vpl* virulence plasmid in the vaccine isolate (Kennedy et al., 1999). Regardless the mechanism, the ability to utilize a commercial serologic kit to evaluate exposure to field *Salmonella* in vaccinated animals would be a valuable epidemiologic tool.

Discussion: Several studies have suggested that vaccination with an avirulent live *Salmonella* vaccine may allow for cross protection and reduction of *Salmonella* in several species (Fox et al., 1997; House et al., 2001). The results of the three independent studies described further support the following conclusions regarding vaccination of pigs with Enterisol® SC-54:

- a.) Vaccination reduces *Salmonella* prevalence in carcasses at harvest.
- b.) Reduction occurs in all species of *Salmonella*, not just homologous/Group C1 *Salmonellae*.
- c.) Vaccination can be a valuable tool in reducing risk of foodborne disease due to *Salmonella* in pork. Vaccination will not interfere with serologic categorization of farms' *Salmonella* status.

Vaccination may be considered as another potential tool for improving safety of pork by means of reducing the level of salmonella contamination of pork carcasses, and potentially ground or other fresh pork products. Commercial serologic tests may be utilized in conjunction with vaccination to evaluate the success of *Salmonella* reduction programs in swine.

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QUANTIFYING TETRACYCLINE RESISTANCE

O 78

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Summary: This study's goal was to evaluate the impact of sub-therapeutic feeding of chlortetracycline (CTC) on the fecal concentration of tet(C), a gene that confers tetracycline resistance via an efflux mechanism. We developed a real-time quantitative PCR assay to measure the quantity of tet(C) in whole fecal DNA samples. The vast proportion of variability in tet(C) (91%) was associated with differences in concentration between the individual pigs, and there was no significant difference in the copy number of tet(C)/mg of feces between the treatment and control pigs ($p > 0.05$, linear regression, SPSS 11.0.5)

Keywords: real-time quantitative PCR, tet(C)

Introduction: Antimicrobial resistance (AR) research has been predominantly confined to the study

of cultivable bacteria. Although this approach provides pertinent information regarding AR, it is a very limited sample of the normal gastrointestinal flora, which could serve as a reservoir for AR genes. Therefore, tools for detection, quantification and tracking of AR genes are very attractive for understanding the ecology of AR. The goal of this study was to quantify the AR reservoir attributable to tet(C) in the feces of swine fed CTC in the diet as compared to pigs not fed CTC. This was accomplished by using real-time quantitative PCR (Q-PCR) to measure the amount of the tet(C) gene present in DNA extracted from fecal samples.

Materials and Methods: Treatments (50g CTC/ton of feed or no CTC) were assigned to 3 temporally matched barn pairs. Fecal samples (200 mg) were collected from 48 pigs per barn. Total fecal DNA was isolated using the QIAamp DNA Stool Mini Kit. From one barn pair, DNA was extracted from each individual pig fecal sample in triplicate to assay the variability of tet(C) concentration within a fecal sample. Quantification of tet(C) in the fecal DNA was done using the Qiagen QuantiTect SYBR Green PCR Kit on a BioRad iCycler iQ Real-Time Detection System with tet(C) primers (Aminov, 2002). A linear regression model was constructed to assess the effect of treatment and farm on tet(C) copy number. A multilevel linear model was constructed to assess the effect of treatment and hierarchical sources of variation in tet(C) concentration for the replicate fecal samples (MLwiN 2.1a, London).

Results: Figure 1A is a representative amplification plot of the samples from real-time Q-PCR assays for tet(C) conducted using the BioRad iCycler iQ Real-Time Detection System. The plot displays the relative fluorescence of double stranded DNA for representative wells at every amplification cycle. Figure 1B is the standard curve, spanning 10^6 to 10^2 copies of tet(C), all samples run to date fall within this range, allowing accurate calculation of copies of tet(C)/mg of feces.

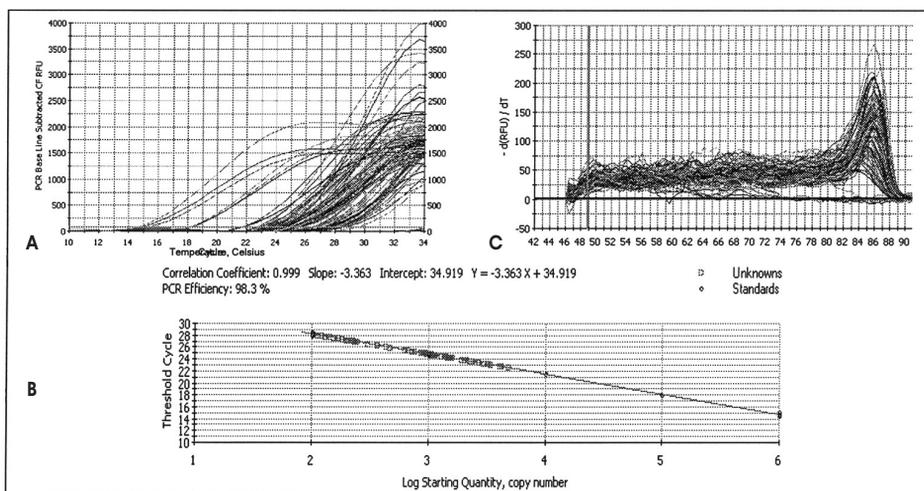


Figure 1. A. PCR Quantification screen output from the BioRad iCycler iQ Real-Time Detection System for representative samples for quantification of tet(C) in pig fecal samples. B. Standard curve of serial 10-fold dilutions of pBR322 from 10^6 to 10^2 copies. Boxes indicate threshold cycles of the fecal samples. The correlation coefficient was 0.999. C. Melt-curve profiles representing products generated from the amplification seen in Figure 1A.

The single prominent peak of the melting curve plot demonstrated in Figure 1C confirms product specificity of the tet(C) primers (expected product T_m of 84-86°C) and also shows no evidence of primer-dimers. The sequence of the tet(C) PCR product has been confirmed by sequence analysis. The greatest proportion of the variability in tet(C) copy number (91%) was associated with differences in concentration in the individual pig, while 4% and 5% of the variation in the copy number estimate was associated with fecal sub-samples and PCR replicates respectively (Fig. 2A). These data are

important as they demonstrate our ability to conduct the real-time quantitative PCR assay, and confirm that the variability in copy number estimates is associated with pig to pig differences in copy number and not variability in the assay technique.

The median copy number was 781 (range 18-5556) for the treatment group and 665 (range 11-6483) for the control group (Fig. 3B). There was no significant difference in the copy number of tet(C)/mg of feces between the treatment and control pigs ($p>0.05$) despite the phenotypic differences previously identified in the Gram-negative fecal flora from these farms (Funk, 2003). Perhaps even more interesting is that Farm 2, which received therapeutic doses of tetracycline during the study and had the highest proportion of Gram-negative fecal isolates resistant to tetracycline overall, does not demonstrate an increase in the copy number of tet(C)/mg of feces relative to farms not receiving therapeutic CTC.

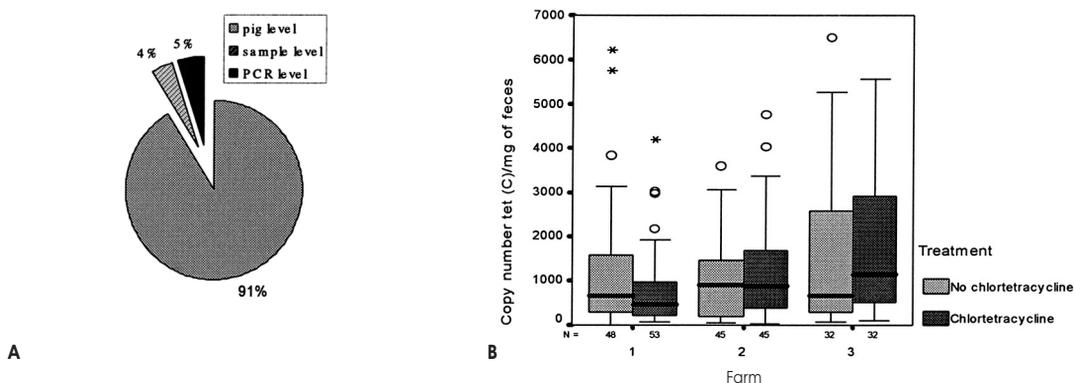


Figure 2. A. Proportion of variability in tet(C) copy number at pig, sample and PCR levels. B. Copy number of tet(C)/mg of feces in pigs receiving CTC in the feed vs. pigs not receiving CTC in the feed.

Discussion: Beyond qualitatively identifying the genes conferring AR present in commensals, there is an important question regarding what measurement of AR reflects the risk of transfer from animals to humans. Although qualitative assays can describe the number of animals that harbor a particular AR gene, it is unknown whether the relative quantity of this gene in an animal is important. In this study there was no detectable effect of short term changes in sub-therapeutic antimicrobial use on tet(C) copy number. Further studies to discern the contribution of other genes that confer tetracycline resistance are on-going.

Acknowledgements: Mike Zianni, MS, manager of the OSU Plant Microbe Genomics Facility.

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Three-Year Trend in Antimicrobial Resistance and Genotypes among *Salmonella* in Swine and Humans

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Summary: The aim of this study was to determine antimicrobial resistance among *Salmonella* isolated from swine and humans in North Carolina, compare genotypes among isolates from humans originated from pig-producing areas and characterize important genes. Resistance to 9 and 11 of the 12 antimicrobial agents tested was detected among isolates from swine and humans respectively. Frequency of resistance to tetracycline and b-lactams was significantly higher among isolates from swine than humans ($p < 0.05$). Two common multi-drug resistance (MDR) patterns were found among isolates from apparently healthy swine: AmKmStSuTe and AmCmStSuTe. However, the former MDR pattern was rare among clinical isolates. Genotyping revealed that two predominant genotypes, one composed of clinical isolates and the other non-clinical were noticed. Further characterization using *Salmonella* plasmid virulence; *spvA* gene also revealed that this gene is absent among the most common MDR pattern, AmKmStSuTe, in swine.

Keywords: pig, antibiotic, fingerprint, food safety, zoonosis

Introduction: North Carolina is the second largest pork-producing state in the United States with more than 10 million pigs marketed each year. Pigs are known to be important reservoirs of *Salmonella* and the use of antimicrobials in food animal production has been implicated as one of the most important source of resistant organisms among human pathogens (Barber et al., 2003). Recent trends also showed that multi-drug resistant strains of *Salmonella* are common among salmonellae from pigs (Gebreyes, 2000; Farrington et al., 1999). Though antimicrobial resistant salmonellae are common among pigs in the region, no human salmonellosis outbreak in humans has been attributed to swine or pork in the last decade in the US. The aim of this study was, therefore, to determine antimicrobial resistance patterns and show whether there is phenotypic and genotypic similarities among isolates from swine and humans and among isolates collected from clinical and non-clinical settings.

Materials and Methods: A total of 888 *Salmonella* isolates of four serovars including Typhimurium, Heidelberg, Muenchen and Newport, from clinical human cases were retrieved from the North Carolina State Laboratory of Public Health (NCCLPH) between the period of 2000 and 2003. In swine, 1873 isolates from on-farm studies (non-clinical) and 65 clinical isolates were included in the study. Antimicrobial susceptibility testing was done using Kirby-Bauer disk diffusion for 12 antimicrobial agents using NCCLS standards [M31-A2]. The antimicrobials, respective codes used in this manuscript and breakpoints for resistance were amikacin [Ak] (64 mg/ml), amoxicillin/ clavulanic acid [Ax] (32 mg/ml), ampicillin [Am] (32 mg/ml), cefotaxime [Cf] (32 mg/ml), cephalothin [Ce] (32 mg/ml), chloramphenicol [Cm] (32 mg/ml), ciprofloxacin [Cip] (2 mg/ml), gentamicin [Gm] (16 mg/ml), piperacillin [Pi] (32-64 mg/ml), tetracycline [Te] (16 mg/ml) and trimethoprim-sulfamethoxazole [Tr/Su] (80 mg/ml). Pulsed-Field Gel Electrophoresis (PFGE) was used for genotyping isolates from human and swine collected at different settings: clinical and non-clinical. The specific protocol used for genotyping was as recommended previously (Gautom, 1997). Further molecular characterization was done by using polymerase chain reaction (PCR) of *Salmonella* plasmid virulence, *spvA* gene.

Results: Frequency of resistance to the most commonly used antimicrobials such as tetracycline and b-lactams was higher among isolates from swine than humans ($p < 0.05$). However, the spectrum of resistance was wider among isolates from humans as indicated by the fact that resistance to 11

of the 12 antimicrobials was detected including ceftriaxone and ciprofloxacin. Among swine isolates, resistance to nine of the antimicrobial agents was detected. The frequency of resistance to each antimicrobial is as shown in figure 1. Comparison of serovar composition and antimicrobial resistance frequency of human isolates from pig-producing (eastern part of the state) and non pig-producing areas (western part of the state) of the region did not reveal any phenotypic difference in composition of serovars nor frequency of antimicrobial resistance ($p > 0.1$). While more than 80% of isolates from humans remained susceptible to all antimicrobial agents tested, subsets of the remaining, particularly, Typhimurium isolates exhibited AmCmStSuTe resistance pattern. Among isolates from swine (non-clinical on-farm samples), two distinct penta-resistance patterns, AmKmStSuTe and AmCmStSuTe, were common. On the other hand, clinical isolates from swine also showed AmCmStSuTe pattern predominantly similar to human isolates but not AmKmStSuTe pattern. Further characterization using genotypic approaches also confirmed that the clinical isolates, irrespective of the host, were closely related (Figure 2). Analysis of *Salmonella* plasmid virulence, *spvA* gene using PCR revealed that this gene is absent among the predominant on-farm strains with AmKmStSuTe resistance pattern but present among the AmCmStSuTe ones (Figure 3).

Figure 1. Frequency of antimicrobial resistance among Salmonella isolates

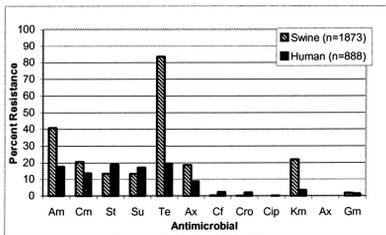


Figure 3. Analysis of *spvA* gene among MDR Salmonella serovar Typhimurium isolates from swine.

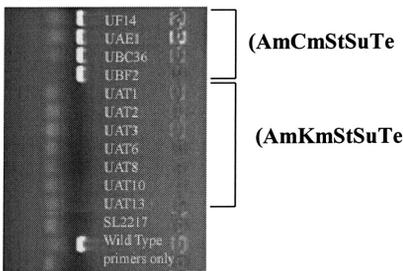
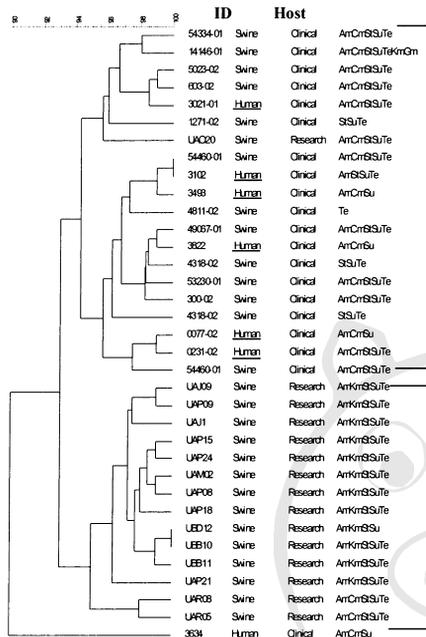


Figure 2. Genetic relatedness of Salmonella serovar Typhimurium isolates from swine (clinical and on-farm) and human (clinical) isolates using PFGE.



Discussion and Conclusions: Frequency of antimicrobial resistance among *Salmonella* isolates from swine was higher than those from humans. This finding is consistent with reports of previous studies (Cruchaga et al., 2001). One important aspect of the findings, however, was detection of ceftriaxone and ciprofloxacin resistance among isolates from humans but not from swine. One potential explanation for this finding may be the high and possibly irrational use of these antimicrobials in humans or could also be due to sampling error. These findings also show that there is strain distinction between the two hosts. Serovar Typhimurium was the predominant serovar in both host species. However, further phenotypic characterization revealed that the most common MDR pattern in apparently healthy pigs, AmKmStSuTe, is absent among human isolates and clinical swine isolates. This may have two

implications. First, it implies that there is distinction in subtypes between the two hosts. This may further imply that other sources may also be important. Recently, report by Barber et al. stressed the underestimation of the role of non-food animal sources such as humans and pets (Barber et al., 2003). Second, the finding may indicate that only certain subtypes of this serovar are able to cause clinical illness strengthening the notion that not all strains of *Salmonella* serovar Typhimurium are capable of causing clinical illnesses.

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O 80

ANTIMICROBIAL AGENT SUSCEPTIBILITY OF CAMPYLOBACTER AND SALMONELLA FROM SWINE HERDS WITH VARIOUS THERAPEUTIC REGIMENS

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Summary: Fecal samples were collected in pens from 27 farrow-to-finish swine herds using (n = 10) and not using fluoroquinolones (n = 17) and in herds using (n = 20) or not (n = 7) tetracycline. Workers on the farms participated to the study. *Salmonella* was found in 4 out of the 27 sampled herds and were all resistant to tetracycline and susceptible to enrofloxacin. No *Salmonella* and no *Campylobacter* were isolated from human. *C. coli* was found in all sampled herds with an average of 68,5 % positive pens. In farms not using quinolones and tetracycline, no resistance was observed among 2 herds but resistance to enrofloxacin (71 %) and to tetracycline (100 %) were observed in some farms. In herds where tetracycline was used, resistance levels varied from 7 % to 100 %. Resistance levels to quinolones in herds using this agent varied from 0 % to 100 %.

Keywords: resistance, agar dilution, fluoroquinolones, tetracycline, antimicrobial use

Introduction: *Campylobacter* and *Salmonella* are zoonotic pathogens frequently recovered from pigs. The incidence of antibiotic resistance of *Campylobacter* and *Salmonella* isolates increased over the past few years. The use of antibiotics in food animal productions would be in part responsible for the increased resistance among foodborne bacterial pathogens (McEwen & Fedorka-Cray, 2002).

Resistance to fluoroquinolones is recognized as an emerging public health problem (Engberg et al., 2001). Recent use of this antimicrobial agent in food animals production would be responsible for this increase in resistance levels. On the other hand, tetracyclines have been and are still widely used to prevent or to treat human and animal infections and as growth promoters in animals (Chopra & Roberts, 2001). The aims of this study were to evaluate the prevalence of *Campylobacter* and *Salmonella* in humans and animals in swine herds and to compare the resistance levels of bacteria recovered from farms using or not antimicrobial agents such as tetracycline and fluoroquinolones.

Materials and Methods: A total of 27 farrow-to-finish farms were sampled once from June to August 2002. Farms were selected as follows: 7 farms not using tetracycline, 20 farms using tetracyclines, 10 farms using fluoroquinolones and 17 farms not using quinolones. At the finishing stage, 10 pens per farm were randomly selected and a pool of 5 g of feces was collected. Cary-Blair swabs were used for collection of human samples. For *Campylobacter* detection, fecal swabs were inoculated onto charcoal-based selective medium (CSM) with supplements and incubated at 42 °C under microaerophilic atmosphere for 48 h (Karmali et al., 1986). From each plate, three typical colonies were processed for biochemical identification tests. For *Salmonella* detection, samples were enriched in nutrient broth for 24 h and 1 ml was transferred into TBG broth for another 24 h at 42 °C. A loopful was plated on BGS media with novobiocine for 48 h. Typical colonies were tested by TSI, urea and slide agglutination for *Salmonella* and sent for serotyping by Health Canada (Guelph, Canada). Resistance to antimicrobial agents tetracycline and enrofloxacin was evaluated by the agar dilution technique for *Campylobacter* and by disks diffusion for *Salmonella*. Breakpoint values were designated as recommended by NCCLS for veterinary pathogens.

Results: *Campylobacter* isolates were found in all sampled herds. All *Campylobacter* were identified as *C. coli* and the incidence per farm varied from 10 % to 100 % of pens with an average of 68,5 % of positive pens. Resistance levels to quinolones in herds using this agent varied from 0 % to 100 % with an average resistance in pens of 24 %. Resistance levels as high as 59 %, 71 % and 75 % were observed among herds from farms using no quinolones. In herds where tetracycline was used, resistance levels to this antimicrobial agent varied from 7 % to 100 %. At the opposite, only 2 out of 7 herds where there was no use of tetracycline had no resistant bacteria. In this study, 4 out of the 27 (14,8 %) sampled herds were positive for *Salmonella*. From these herds, various serotypes, including *S. Thyphimurium* DT104, were observed in a maximum of 30 % of pens on a given farm. All *Salmonella* isolates were resistant to tetracycline and susceptible to enrofloxacin. They all came from farms using no quinolone. However, tetracycline was used on 1 out of 4 positive herds. No *Salmonella* and no *Campylobacter* were isolated from human samples.

Discussion: In this study, resistant *Campylobacter* were recovered more frequently in pens from farms not using quinolones than in farms using this drug. On the other hand, even if tetracycline is widely used in swine production, in some farms not using this product, no resistance to this antimicrobial agent was observed in *Campylobacter* isolates from those herds. A previous utilization in another production cycle and/or the transmissible nature, for tetracycline, of the resistance determinant may explain the recovery of resistant isolates in farms not using quinolones and/or tetracycline. Prevalence of *Salmonella* was relatively low compared to other studies. The low recovery rate for *Salmonella* in this study make it hard to conclude on the impact of antibiotics use on a possible selection of resistant *Salmonella* isolates. In addition the absence of *Salmonella* and *Campylobacter* in all human samples suggest that few transmissions from animals to humans occurred in these herds. Overall, our findings indicate that the resistance to antimicrobial agents such as fluoroquinolones and tetracycline in *Campylobacter* isolates found on swine farms is weakly associated with the on farm use of these antimicrobial agents.

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O 81 ANTIMICROBIAL AGENTS RESISTANCE IN *CAMPYLOBACTER COLI* FROM SWINE AND HUMANS

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Summary: *C. coli* from slaughtered pigs and from human patients were examined for resistance to quinolones and tetracycline. Detection of *tetO* was performed. Gyrase A gene (*gyrA*) was amplified and sequenced and tested by an alternative method. Tetracycline resistance levels were respectively of 67,7 % and 56,3 % in swine and human isolates. In *C. coli* of human origin, all resistant isolates had the *tetO* determinant while 82,8 % of resistant *C. coli* from swine possessed it. Among the susceptible swine isolates, 17,2 % possessed this gene. Resistance to enrofloxacin (7,3 %) and ciprofloxacin (11,4 %) was observed in swine isolates and resistance to enrofloxacin (12,5 %) and ciprofloxacin (18,8 %) were observed in *C. coli* from humans. In addition, 72,7 % of swine resistant isolates and all isolates from humans had a mutation at position 86. Results were similar with MAMA-PCR which can thus be considered as a good alternative to sequencing.

Keywords: MAMA PCR, *tetO*, *gyrA*, agar dilution, sequencing.

Introduction: An increase in antimicrobial agents resistance have been reported in many countries for *Campylobacter*, specially for tetracycline, fluoroquinolones and erythromycin (Engberg et al., 2001). *Campylobacteriosis* is generally associated with sporadic episodes of diarrhea linked with consumption of improperly handled or cooked food. Animal productions such as swine are potential reservoirs for this bacteria. Some authors suggested that the usage of antimicrobial agents in animal productions play a key role in the dissemination of antimicrobial resistance genes from animals to human population (Swartz 2002). To evaluate this possible link it is important to verify the distribution of antimicrobial resistance determinants in various populations. The aim of this study was to evaluate the incidence and the distribution of antimicrobial resistance in *Campylobacter* isolates from humans and swine recovered within a restricted geographical area in order to increase chances to establish links. The detection of some genetic determinants of resistance was performed in order to characterize the resistance.

Materials and Methods: A total of 16 human clinical cases isolates and 96 isolates from cecal content of slaughtered swine were included in the study. Resistance tetracycline and fluoroquinolones was evaluated by the agar dilution technique following guidelines of the NCCLS for veterinary pathogens.

Following DNA extraction, *tetO* gene was amplified by PCR using primers previously described (Widdowson et al., 1996). A PCR product containing the quinolone resistance-determining region (QRDR) was generated to be further sequenced (Zirstein et al., 2000) at Sheldon Biotechnology Center, McGill University, Montreal, Canada. The Mismatch amplification mutation assay-PCR (MAMA-PCR) was used to evaluate the reliability of this technique as an alternative to sequencing. A conserved forward primer and a reverse mutation detection primer were used for *C. coli*. As expected, a 192-bp PCR product for *C. coli* was a positive indication of the presence of the Thr-86 to Ile mutation in *gyrA* gene.

Results: There was no significant difference between resistance levels to ciprofloxacin and enrofloxacin from swine and humans isolates and the highest resistance level observed was for tetracycline (Table 1). The genetic determinant *tetO* was recovered from 82,8 % of swine resistant isolates and from 100 % of the resistant human isolates. On the other hand, 17,2 % (5/29) of the swine susceptible isolates had the *tetO* determinant. After the sequencing of quinolones resistant *C. coli*, all human isolates and 7 out of 11 (63,6 %) of swine isolates had the transition of one nucleotide associated with a change from a threonine to an isoleucine at position 86 of *GyrA*. These findings were in accordance with results obtained by MAMA PCR since the wild-type amino acid 86 codon was not amplified with the reverse mutation primer. No other amino acid substitution was observed.

Table 1. Antimicrobial agents susceptibility of *C. coli* isolated from swine and humans

| Antimicrobial agent | Pigs (n = 96) | | | | Human (n = 16) | | | |
|---------------------|--------------------------------|-------------------|-----------|-----------------|-------------------|-------------------|-----------|------|
| | MIC ₅₀ ^a | MIC ₉₀ | range | %R ^b | MIC ₅₀ | MIC ₉₀ | range | %R |
| Ciprofloxacin | <0,25 | 0,5 | <0,25-32 | 11,4 | 16 | | <0,25-16 | 18,8 |
| Enrofloxacin | | <0,25 | <0,25-16 | 7,3 | <0,25 | | <0,25-8 | 12,5 |
| Tetracycline | 32 | 64 | <0,25-128 | 67,7 | 1 | 64 | <0,25-128 | 56,3 |

^aMIC in µg/ml

^b%R, percentage resistant

Discussion: Tetracyclines are relatively inexpensive drugs with a broad spectrum of activity and have been widely used. The highest levels of resistance observed were for this antimicrobial agent. Similar results were observed in studies conducted in Belgium (Van Looveren et al., 2001) and Italy (Pezzotti et al., 2003). So far, *tetO* is the only gene reported to be responsible for the resistance in *Campylobacter*. In this study, 17,2 % of the tetracycline susceptible swine isolates possessed the determinant but did not express it. A required DNA sequence upstream of this gene (Wang & Taylor, 1991) might have been altered in those isolates, affecting their expression. Other isolates, found resistant by the agar dilution technique, did not possess *tetO* suggesting that other genetic determinant might be present. Resistance to fluoroquinolones in *Campylobacter* from food animal origin is recognized as an important emerging public health threat (Engberg et al., 2001). Mutations in *gyrA* at positions Thr-86, were reported as mainly responsible for quinolone resistance. In this study, only the Thr-86 mutation have been observed. Since some isolates that did not possessed the mutation in *gyrA* had high MIC, it suggest that the resistance could be linked to others genes, such as *gyrB*, topoisomerase IV *parC* and *parE*, or to efflux pumps or permeability factors.

Acknowledgments: We gratefully acknowledge Bayer Inc for quinolones supplies and the CFIA's Laboratory of Food and Veterinary Hygiene for access to their facilities.

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FAECAL SHEDDING OF ARCOBACTER SPECIES IN BELGIAN PIGS

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Summary: The prevalence of *Arcobacter* was determined in porcine faecal samples collected at slaughterhouse and two unrelated finishing barns (A and B) using the previously developed *Arcobacter* isolation procedure. In 43.9% of the slaughterhouse samples tested (n=82) arcobacters were detected, and identified as *A. butzleri* and *A. cryaerophilus*. Two pigs shedded both species simultaneously. On farm A (n=98), arcobacters were isolated from 16.3% of the samples and identified as *A. cryaerophilus* and *A. skirrowii*. In samples (n=118) collected at farm B, arcobacters were detected in 45.1% of the samples. *A. butzleri* was the most frequently occurring species. Co-infections were found in 11 animals. Arcobacters were detected in clinically healthy pigs at contamination levels up to 10³ cfu/g faeces.

Keywords: Porcine faeces, *Arcobacter* prevalence, Slaughterhouse, Farm level, Belgium.

Introduction: The genus *Arcobacter* includes bacteria formerly known as aerotolerant campylobacters. They are Gram-negative non-spore-forming rods with a single polar flagellum and differ from the closely related campylobacters in their ability to grow aerobically from 15 up to 42 °C. Within the genus *Arcobacter*, four species are presently recognized: *Arcobacter nitrofigilis*, a nitrogen-fixing plant associated species (McClung et al., 1983) and the animal and human related species *Arcobacter butzleri*, *Arcobacter cryaerophilus* and *Arcobacter skirrowii*. Arcobacters are frequently present on foods of animal origin, including pork (Collins et al., 1996). Contamination probably occurs by faecal contamination during slaughter. Foods of animal origin are considered as a main source of *Arcobacter* infection in humans and therefore, arcobacters are established as an emerging foodborne pathogen (Wesley, 1996). In pigs, arcobacters are associated with reproductive problems and are found in stomachs of pigs with gastric ulcers. Moreover, arcobacters can be isolated from faeces of clinically healthy animals.

The aim of the present study was to determine the prevalence and contamination level of *Arcobacter* in Belgian pigs at slaughter age and during raising.

Materials and Methods: Porcine faecal samples were rectally collected at the slaughterhouse (n=78) and at two unrelated finishing farms (n₁=98; n₂=118). On farm B, samples were also collected from available water sources (n=7) and from the sole on the farmers boots (n=1). One g faeces per sample was transferred into a stomacher bag. Next, nine ml *Arcobacter* Selective Isolation Broth supplemented with 50 ml/l lysed defibrinated horse blood was added (Van Driessche et al., 2003). The mixtures were homogenized with a stomacher blender and 100 ml of each homogenate was brought onto *Arcobacter* Selective Isolation Agar (ASIA) plates by the spiral plating method (Van Driessche et al., 2003). The remaining homogenates were incubated for 48 h at 28°C. Following incubation, 50 ml of the enrichment was streaked onto ASIA plates. Plates were incubated for 24 to 72 h at 28°C and checked every 24 h for bacterial growth. All colonies were counted, picked and subcultured onto blood agar plates. Bacterial growth was harvested and identified at species level by a multiplex-PCR assay (Houf et al., 2000).

Results: From the 82 slaughterhouse samples, *A. butzleri* was isolated from 29 and *A. cryaerophilus* from seven samples after enrichment. Twenty-three of the 35 pigs were also positive by direct isolation, 18 of which with *A. butzleri* and three with *A. cryaerophilus*. Two pigs had a co-infection with both species. From the collected samples at farm A, six of the 98 had a bacterial load of more than 10² arcobacters/g faeces. Arcobacters were isolated from ten additional animals by the enrichment procedure. *A. cryaerophilus* was isolated from 14 pigs. Two animals shedded *A. cryaerophilus* and *A. skirrowii* simultaneously. From the samples (n=118) collected at farm B, arcobacters were isolated from 20 pigs at levels of 10² to 10³ cfu/g faeces. Additionally, 29 animals tested positive after enrichment. *A. butzleri* was isolated from 32 pigs, *A. cryaerophilus* from 2 animals and *A. skirrowii* was found in 4 animals. Furthermore, 11 pigs had a co-infection with more than one species. From the 7 water samples, arcobacters were isolated from five samples using enrichment. *A. butzleri* was found in two samples, *A. cryaerophilus* in one and *A. butzleri* and *A. cryaerophilus* were present simultaneously in two samples. From the sole on the farmers boots, *A. cryaerophilus* was isolated by direct isolation and *A. butzleri* by enrichment.

Discussion: In the present study, the prevalence of *Arcobacter* in porcine faeces at slaughterhouse level was 43.9%. In a recent study, 10% of the examined faecal samples (n=250) of pigs in a Japanese abattoir were found positive for *Arcobacter* (Kabeya et al., 2003). In our study at farm level, prevalences of 16.3% and 45.1% were obtained. Hume et al. (2001) found on a farrow-to-finish swine facility in Texas 36.3% of sows examined (n=55) positive for the presence of *Arcobacter*. Difference in prevalence may be caused by the different isolation protocols used, the sampling method, the season, the regional influences, farm management, and animals' age.

Conclusions: In the present study, arcobacters were detected in clinically healthy Belgian pigs at levels up to 10³ cfu/g faeces. Animals can excrete different *Arcobacter* species simultaneously.

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O 83 Mycobacterial contamination of environment in pig farms in the Czech Republic between 1996 and 2002

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Summary: The purpose of this study was to find source of mycobacterial infections in pig farms. A total of 2 411 environmental samples (bedding materials, water, biofilm from pipelines, peat, etc.) were examined by microscopy and culture. Isolates were identified by serotyping and PCR. Mycobacteria were isolated from 579 (24.0%) samples. 47.0% isolates were *Mycobacterium avium* subsp. *hominissuis* isolates (IS901-, IS1245+, serotypes 4, 6, 8, 9), 2.2% isolates were *M. a. avium* (IS901+, IS1245+, serotype 2) and 50.8% belong to atypical mycobacteria comprising of fifteen species. The frequent isolates were found in peat samples (213/65.1%) in which 81.2% isolates comprised *M. a. hominissuis*. High amount of mycobacteria were isolated from biofilm (36.4%) and water (29.6%). Alike peat, non-pathogenic species were predominant. The third sources of mycobacteria were bedding materials, mostly sawdust (43.6%). Presence of mycobacteria in the animals' environment leads to economic losses due to meat condemnation in abattoirs.

Keyword: *Mycobacterium avium* complex, mycobacteriosis, peat, water, bedding

Introduction: Among domestic animals pigs are highly sensitive to mycobacterial infection. Tuberculous lesions in pigs involve mainly the head and/or the mesenteric lymph nodes (Thorel *et al.*, 1997). Occurrence of tuberculous changes in the lymph nodes at abattoirs leads to confiscations of pork meat and organs or their assessment as conditionally comestible. The main agents causing infection in pigs are the group of mycobacteria referred to as *Mycobacterium avium* complex (MAC). Serotypes 4-6, 8-11 and 21 belonging to *M. a. hominissuis* are the most frequently isolated from pigs (Komijn *et al.*, 1999). Epidemiological studies on mycobacterial lymphadenitis indicate that the environment may represent a possible source of infection (Gardner and Hird, 1989). The main environmental sources of mycobacteria comprise beddings, feed, water and peat. The aim of this study was to assess the impact of environmental mycobacteria in relation with finding of gross tuberculoïd alterations on head and mesenteric lymph nodes.

Materials and Methods: A total of 2 411 samples from inner and outer stable environment (Table 1) were originated from 50 farms of 29 districts. Bedding materials constituted straw, hay, silage, sawdust and tree bark. Smears made from specimens were examined by direct microscopy according to Ziehl-Neelsen staining. All samples were decontaminated by 1N HCl and 2N NaOH. The resuspended samples were inoculated onto Herrold's, Stonebrink and Sula medium and incubated at 25° and 37°C, respectively. Growth of mycobacteria was evaluated after one week of incubation and then each two weeks. Isolates of mycobacteria were identified by IS901 and IS1245 PCR, serotyping and biochemical tests.

Results: Results of microscopic and culture examination and identification of isolated mycobacteria from different environmental material is shown in Table 1.

Table 1. Examination of environmental materials from pig farms and identification of isolated mycobacteria

| Biological samples | Positive examination by: | | Mycobacterial isolates | | | | | | | | | | |
|---------------------------------|--------------------------|----|------------------------|-----|---------|----|--------------------|--------------------------|--------------------|---------------------|--------------------|--------------------|---------------|
| | | | Microscopy | | Culture | | <i>M. a. avium</i> | <i>M. a. hominissuis</i> | <i>M. gordonae</i> | <i>M. fortuitum</i> | <i>M. chelonae</i> | <i>M. favesces</i> | other species |
| | | | No. | % | No. | % | | | | | | | |
| Peat | 327 | 10 | 3.1 | 213 | 65.1 | 3 | 173 | 2 | 3 | 1 | 1 | 30 | |
| Concentrates | 270 | 2 | 0.7 | 28 | 10.4 | 0 | 12 | 1 | 2 | 0 | 1 | 12 | |
| Kaolin and char coal | 116 | 0 | 0 | 10 | 8.6 | 0 | 2 | 0 | 0 | 0 | 0 | 8 | |
| Biofilm from pipelines | 217 | 59 | 27.2 | 79 | 36.4 | 0 | 2 | 36 | 12 | 3 | 7 | 19 | |
| Water from pipelines | 233 | 9 | 3.9 | 69 | 29.6 | 0 | 17 | 22 | 9 | 2 | 4 | 15 | |
| Bedding | 231 | 7 | 3.0 | 61 | 26.4 | 7 | 24 | 0 | 5 | 1 | 0 | 24 | |
| Pig faeces | 179 | 5 | 2.8 | 28 | 15.6 | 0 | 10 | 1 | 2 | 1 | 0 | 14 | |
| Stable scrapings | 174 | 1 | 0.6 | 32 | 18.4 | 1 | 12 | 1 | 2 | 0 | 1 | 15 | |
| Dust and spider nets | 117 | 2 | 1.7 | 9 | 7.7 | 0 | 3 | 0 | 2 | 0 | 0 | 4 | |
| Soil from the paddocks | 19 | 0 | 0 | 3 | 15.8 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | |
| Free living birds, birds' nests | 80 | 0 | 0 | 12 | 15.0 | 2 | 1 | 1 | 3 | 1 | 0 | 4 | |
| Small terrestrial mammals | 19 | 0 | 0 | 1 | 5.3 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | |
| Non-vertebrates | 430 | 0 | 0 | 34 | 7.9 | 0 | 16 | 0 | 6 | 8 | 0 | 4 | |
| Total | 2 412 | 95 | 3.9 | 579 | 23.8 | 13 | 272 | 64 | 47 | 17 | 14 | 152 | |

Concerning bedding material, the most frequent mycobacteria were isolated from sawdust (43.6%), hay (22.2%), straw (13.5%) and bran (10.8). Besides *M. a. hominissuis*, *M. a. avium* isolates were prevalent in sawdust (17.6%). Four different mycobacterial species were isolated from organs of three birds caught in the barn near vicinity. Apart from mycobacterial species shown in Table, *M. terrae* (1.9%), *M. phlei* (1.2%), *M. scrofulaceum* (1.2%), *M. diernhoferi* (0.5%), *M. smegmatis* (0.5%), *M. triviale* (0.5%), *M. xenopi* (0.3%), *M. intracellulare* (0.3%), *M. szulgai* (0.2%), *M. gastri* (0.2%) and *M. ulcerans* (0.2%) were detected. The rest 19.2% isolates were not identified, but they did not belong to MAC.

Discussion: Peat was found the most contaminated material by mycobacteria. The use of peat as a food supplement for piglets induced development of tuberculous lesions in pigs' lymph nodes (Pavlik *et al.*, 2003). High presence of MAC can be explained by further contamination during mining and processing of this material by faeces of birds, small terrestrial vertebrates (Kazda, 2000). The second very important source of mycobacteria were proved bedding materials. The importance of this material is attributed to the dominance of *M. a. avium* (serotype 2) in sawdust. The importance mycobacterial contamination of water and biofilm by conditionally pathogenic mycobacteria cannot be overlooked. A high presence of atypical mycobacteria in such condition may complicate tuberculin skin test and serological tests as a result of cross reaction and in some cases can lead to creation of tuberculous lesions in lymph nodes (Bercovier and Vincent, 2001). In order to prevent tuberculous lesions in pigs, the use of such materials representing a potential source of mycobacteria should be avoided.

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O 84

Survival of *Salmonella* and *Escherichia coli* in pig slurry: results of a plot study

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Summary: Application of slurry from swineherds infected with multi-resistant *Salmonella* Typhimurium DT104 (MRDT104) to arable land is considered a hazard for transmission to wildlife and farm animals, and in Denmark slurry from such herds must be ploughed in. We investigated the effect of 4 different application methods on survival of *Salmonella* and *E. coli* in a plot study. Both organisms could not be detected at any time following ploughing in of contaminated slurry. Following harrowing, injection and hose application, *Salmonella* and *E. coli* could not be detected after 7 and 21 days, respectively. The results suggest that alternative methods may be considered for application of MRDT104 slurry.

Keywords: ploughing, harrowing, slurry injection, hose application, bacterial decay.

Introduction: In Denmark, slurry from herds infected with multi-resistant *Salmonella* Typhimurium DT104 (MRDT104) has to be ploughed in according to current regulations. This decision is based on reports suggesting that MRDT104 is more hazardous to human health than other *S. Typhimurium*. An unsolved question at the time the decision was taken was to which extent this costly measure could actually reduce the risk of transmission to wildlife and neighbouring farms. An MRDT104 infected swineherd can only get exemption from ploughing in if it has a low (<0.1 cfu/g) or moderate (<1 cfu/g) infection level. To assess alternative ways of slurry disposal, we carried out a plot study to determine the survival of MRDT104 in slurry from MRDT104 infected swine herds applied to arable land.

Materials and Methods: The study was carried out in Denmark in an MRDT104-infected swine herd during spring 2002. Eight samples were taken from the slurry tank prior to spreading. Subsequently, slurry was spread on farmland in four ways: (1) hose applicator on black soil followed by ploughing and harrowing; (2) hose applicator on pre-ploughed, black soil followed by harrowing; (3) hose applicator on a field with winter wheat without further soil treatment; (4) slurry injection into soil on a field with winter wheat. Following spreading, eight soil samples were collected from each plot on

day 0, 7, 14, 21, and 28, and analyzed for *Salmonella* (qualitatively, detection limit log 0 cfu/g) and *E. coli* (quantitatively, detection limit log 1 cfu/g) using standard bacteriological methods.

Table 1. Detection of *Salmonella* Typhimurium DT104 and *Escherichia coli* in soil over time following application of pig slurry on farm land by four different methods (number of positive samples/total).

| Application method | Day 0 | Day 7 | Day 14 | Day 21 | Day 28 |
|-----------------------|-------|-------|--------|--------|--------|
| <i>Salmonella</i> | | | | | |
| Ploughing + harrowing | 0/8 | 0/8 | 0/8 | 0/8 | 0/8 |
| Harrowing | 3/8 | 0/8 | 0/8 | 0/8 | 0/8 |
| Injection into soil | 1/8 | 1/8 | 0/8 | 0/8 | 0/8 |
| Hose application | 4/8 | 0/8 | 0/8 | 0/8 | 0/8 |
| <i>E. coli</i> | | | | | |
| Ploughing + harrowing | 0/8 | 0/8 | 0/8 | 0/8 | 0/8 |
| Harrowing | 8/8 | 5/8 | 6/8 | 2/8 | 0/8 |
| Injection into soil | 7/8 | 8/8 | 4/8 | 6/8 | 0/8 |
| Hose application | 8/8 | 5/8 | 5/8 | 2/8 | 0/8 |

Results: Pre-spreading soil samples collected from all four fields were negative for both *Salmonella* and *E. coli*. All pre-spreading slurry samples collected from the tank were positive for *Salmonella* (0.2 cfu/g) and *E. coli* (log 5 cfu/g). Neither *Salmonella* nor *E. coli* was detected on the plot that was ploughed in following slurry application. *Salmonella* was only detected on day 0 (8/32 positive) and in 1 sample on day 7 (Table 1). *E. coli*, which was used as an indicator organism, was detected on day 0, 7, 14 and 21, but no longer on day 28 (Table 1). Quantitative analysis of *E. coli* data showed that there were only minor differences in bacterial decay between the three remaining application methods (see Fig. 1). By day 28, the number of *E. coli* bacteria in the soil had fallen below the detection limit for all application methods.

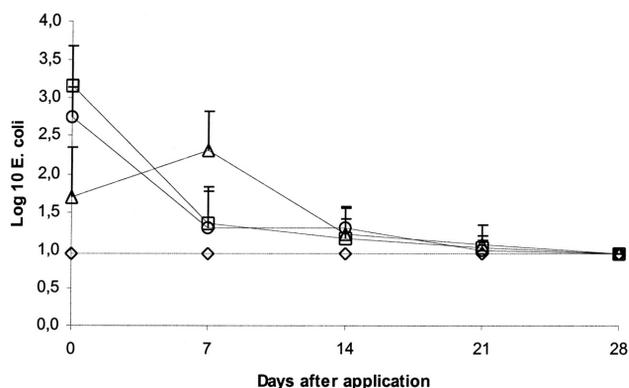


Figure 1. Survival of *E. coli* (mean cfu/g + SD) in pig slurry applied to farm soil following ploughing and harrowing (□), harrowing only (△), slurry injection (○), or hose application without further soil treatment (◇). Detection limit for *E. coli* is log 1 cfu/g.

Discussion and conclusion: The *Salmonella* level observed in this study was comparable to that in the majority of 62 Danish swineherds infected with MRDT104 in 2001/2002. Less than 15% of these herds had high *Salmonella* levels (>110 cfu/g, determined by the Most Probable Number method), suggesting that *Salmonella* levels in naturally contaminated swine slurry are low. This is in accordance with previous Danish investigations. The results showed a rapid decline of both *Salmonella* and *E. coli*.

coli levels, suggesting that alternative methods of slurry application may be considered in MRDT104 infected swineherds, regardless of infection level. The different pattern of decay for slurry injection is due to a sampling error on day 0. Linear extrapolation suggests that the actual *E. coli* counts on this plot on day 0 probably were at least log 3.5 cfu/g. This study also provided input parameters for a simulation model estimating survival of *Salmonella* when slurry from clinically and subclinically infected swineherds is applied to arable land (see Alban & Boes, in this issue).

O 85

Control on the Illegal Use of Clenbuterol in Pigs In Hong Kong

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Clenbuterol is a beta-agonist normally used for the treatment of chronic obstructive pulmonary disease in horses (Adams 1984) and management of parturition in cattle and sheep (Sasse 1987). However, it also has leanness-enhancing effects when included in the feed of several livestock species, including swine (Ricks et al., 1984; Anderson et al., 1990; Mersmann, 1998). Studies on improved growth promotion associated with beta-agonist in feed first appeared in the literature in the early 1980s (Baker and Kiernan 1983, Baker et al. 1984). Significant improvements in food conversion ratio were found in a large range of livestock species, including cattle (Allen et al. 1997), sheep (Hanrahan et al. 1987), pigs (Jones et al. 1985) and poultry (Dalrymple and Ingle 1987).

Clenbuterol has not been approved as a feed additive by many countries in the world because its residues remain in tissues of treated cattle and pigs and resulted in human poisonings (Pulce et al., 1991; Salleras et al., 1995; Sporano et al., 1998). Levels of clenbuterol in animal tissues have been reported to range from 375 to 500 ppb in calf liver (Pulce et al., 1991) to 500 ppb in calf muscle (Maistro et al., 1995). To minimize the potential risk to public health, a maximum permitted residue level of 0.5 ug/kg has been introduced by the European Union (EU) in 1992 (Report, 1992). In North America, the illegal use of clenbuterol came to the attention of meat inspection officials in the United States and Canada in 1988 (Mitchell 1997 CVM). In 1991, the Food Safety and Inspection Service of the US Department of Agriculture announced that it would condemn meat tested positive for clenbuterol residues.

Although many countries have banned the use of clenbuterol as a growth promoter, the economic advantage associated with its use has encouraged the continued abuse of this and other beta-agonists. In 1993, monitoring programmes in EU countries for beta-agonists detected between 0% (UK, France and Denmark) to 7% (Italy) positive samples (Kuiper et al. 1997).

In Hong Kong, clenbuterol hydrochloride is not registered as a pharmaceutical product for use in livestock. However, in May 1998, a number of citizens developed tremor and palpitation after consuming pig offal containing clenbuterol residues. Live pig and meat traders were approached for withdrawal of pig offal from sale. Later in August, a rapid testing programme was introduced in slaughterhouses to screen out pigs fed with clenbuterol coupled with a tattoo identification system to trace pigs to their farms of origin. The system proved to work effectively. Pigs tested positive were detained and could be traced back for further management, including possible prosecution of their owners.

From 1999 to 2002, over nine million live pigs were slaughtered for local consumption in Hong Kong. All pigs must be screened negative for beta-agonists before being slaughtered. Urine samples were

used and subject to using a commercial enzyme immunoassay, with positive results confirmed by testing meat samples using gas chromatography-mass spectrometric analysis.

The numbers of urine samples taken were 56,331, 60,861, 85,318 and 71,716 in the years 1999, 2000, 2001 and 2002 respectively. The percentages of positive samples were 0.98, 0.59 and 0.23 from 1999 to 2001. For pigs with positive results, the whole consignment was detained for investigation. Since August 1998, the rate of contaminated offal at slaughterhouses dropped significantly from 6.6% before the system was implemented, to less than 1% soon after. With the introduction of a new legislation on 31 December 2001 to legalize the above protocol, there were only four (4) positive samples for clenbuterol in 2002.

Very few studies have been published on the total residues remaining in edible tissues of pigs treated with clenbuterol. The period for which drug residues remain detectable in an animal is dependent on the dose administered, the duration of exposure and the persistence in target tissues (Elliott 1994). There is a 15-day withdrawal period following the therapeutic use of clenbuterol, and residues can still be detected in the livers of treated cattle 14 days after administration (Meyer and Rinke 1991). Other studies have also shown that the liver is the target site for detection of clenbuterol residue persistence (Elliott et al., 1993). Therefore, pig meat and offals are also monitored in slaughterhouses and retail outlets for evidence of clenbuterol or salbutamol contamination in case pig farmers try to evade detection by the ante-mortem urine screening system.

14,253 meat and offal samples were taken between 1999 and 2001. 1.3% of 3,638 samples, 1.3% of 4,893 samples and 0.5% of 5,722 samples tested positive for both clenbuterol and salbutamol in the years 1999, 2000 and 2001 respectively. The results showed a marked decrease from 1999 to 2001. The highest levels of clenbuterol recorded in positive offals were 2,000 ppb in lung and 520 ppb in liver in retail shops. However, samples taken at slaughterhouse in 2000 averaged less than 5 ppb. Comparing the surveillance results from slaughterhouses and retail outlets, there was a significant difference in positive samples, with the latter having almost twice as many positive samples. This exposed the suspicion that some retail outlets might have obtained their pork and offal from illegal sources. During 1998-1999, 40 consignments of pigs (340 total) were seized from illegal slaughterhouses, and 70% of such animals tested showed a high positive percentage for clenbuterol in urine. The concentrations of clenbuterol and salbutamol in urine and other internal organs were measured for two batches of pigs seized at local slaughterhouses with positive results. The results (Figs 1a and b) indicated variable concentrations in internal organs of clenbuterol and salbutamol, suggesting that both substances had been used. The liver and lung were the preferable target organs for clenbuterol detection. Another study was performed in four batches of pigs which had tested positive for beta-agonists at slaughterhouses on the time required for excretion of clenbuterol. (Figs 2a, b, c & d). The urine concentration of clenbuterol took only 14 hrs to fall from a detectable level (25 ppb) to undetectable level, using commercial screening tests (Fig 2a). For higher concentrations in urine (Fig 2b), Pig 2 with 45 ppb clenbuterol in its urine required 30 hours to fall to less than 5 ppb which is the usually adopted cut off point for beta-agonist screening so as to avoid false positive cases. In Fig 2c, the concentration of clenbuterol in urine of Pig 2 increased from less than 5 ppb to greater than 52 ppb. This indicated the concentration of clenbuterol in urine may vary with the water intake of the animal, or there are other factors affecting the metabolism of animals fed with clenbuterol. In some cases when pigs were fed excessive amount of clenbuterol (Fig 2d), the urine level remains detectable for 72 hours. The results of these monitoring studies show that illegally treated animals would likely have variable levels of clenbuterol in their urine and that they may not always be identifiable by pre-slaughter screening.

Figure 1a: Studies of concentration of clenbuterol in internal organs and urine of one batch of pigs fed seized at slaughterhouses

| Pig Tattoo Number | Urine $\mu\text{g/kg}$ | Heart $\mu\text{g/kg}$ | Lung $\mu\text{g/kg}$ | Liver $\mu\text{g/kg}$ | Kidney $\mu\text{g/kg}$ | Urinary Bladder $\mu\text{g/kg}$ | Meat $\mu\text{g/kg}$ |
|-------------------|------------------------|------------------------|-----------------------|------------------------|-------------------------|----------------------------------|-----------------------|
| ABCD1 | 1.75 | 0.6 | 4.6 | 3.8 | 2.3 | 9.6 | Not Done |
| ABCD 2 | <1 | N/D | 3.8 | 1.1 | 1.7 | N/D | Not Done |
| ABCD 5 | 11.27 | N/D | 1.5 | 1.8 | N/D | N/D | Not Done |

Figure 1b: Concentration of clenbuterol in internal organs and urine on second batch of pigs seized at slaughterhouse

| Pig Tattoo Number | Drug Type | Urine $\mu\text{g/kg}$ | Heart $\mu\text{g/kg}$ | Lung $\mu\text{g/kg}$ | Liver $\mu\text{g/kg}$ | Kidney $\mu\text{g/kg}$ | Urinary Bladder $\mu\text{g/kg}$ | Meat $\mu\text{g/kg}$ |
|-------------------|-------------|------------------------|------------------------|-----------------------|------------------------|-------------------------|----------------------------------|-----------------------|
| 1 | Clenbuterol | >52 | 5.1 | 68 | 94 | 40 | 10 | N/D |
| | Salbutamol | | N/D | 1.5 | 1.4 | 5.0 | 0.9 | |
| 2 | Clenbuterol | 27.77 | 1.2 | 2.8 | 1.0 | 0.6 | 1.1 | N/D |
| | Salbutamol | | N/D | 10 | N/D | 3.4 | 18 | |
| 3 | Clenbuterol | >52 | N/D | N/D | 1.5 | 0.8 | N/D | N/D |
| 4 | Clenbuterol | <1 | N/D | N/D | N/D | N/D | N/D | N/D |
| | Salbutamol | | N/D | N/D | N/D | N/D | N/D | |
| 5 | Clenbuterol | >52 | N/D | 2.5 | 8.3 | 3.0 | 14 | N/D |
| | Salbutamol | | N/D | N/D | N/D | 0.5 | 4.0 | |
| 6 | Clenbuterol | >52 | N/D | N/D | N/D | N/D | N/D | N/D |
| | Salbutamol | | N/D | 6.0 | N/D | N/D | N/D | |

Figure 2a

Clenbuterol Testing of Pigs of Tattoo no. XXXX

Clenbuterol conc. (ng/ml)

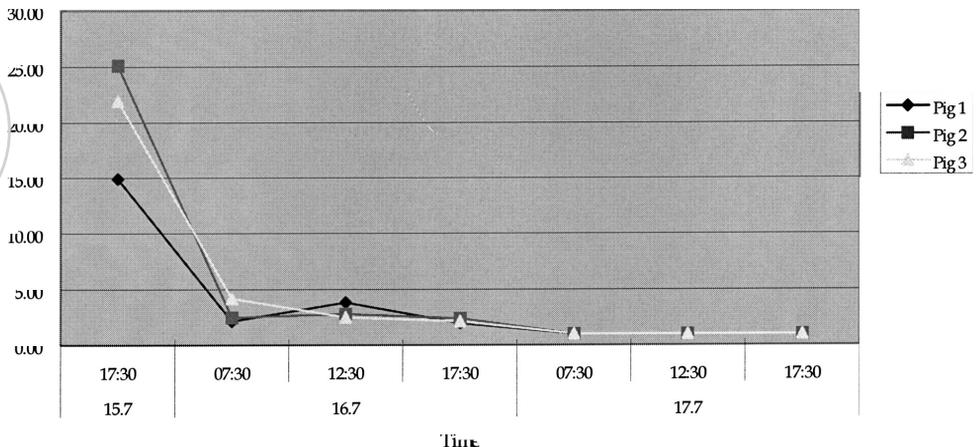


Figure 2b

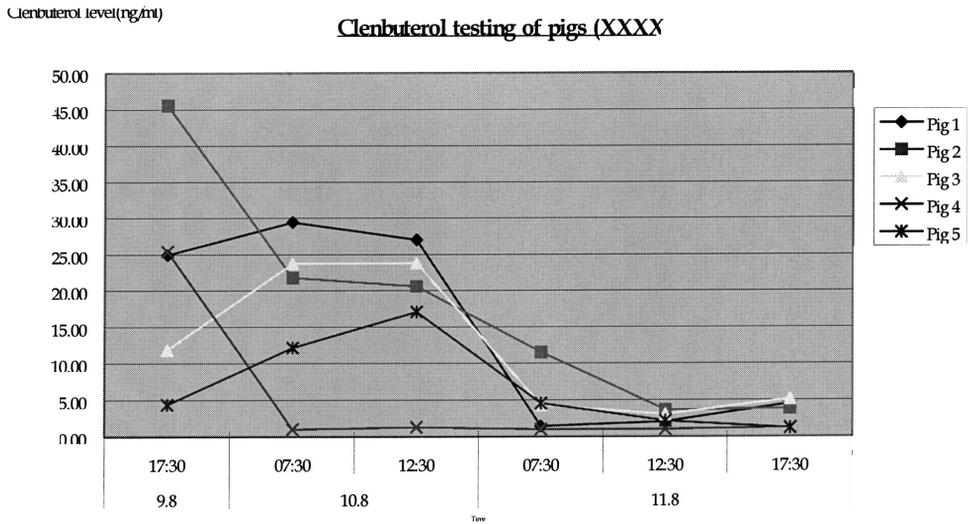


Figure 2c

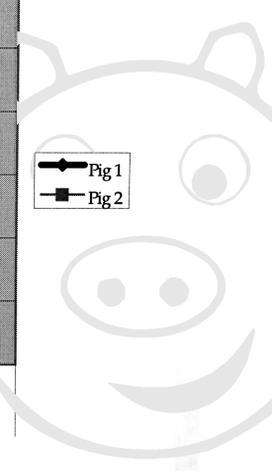
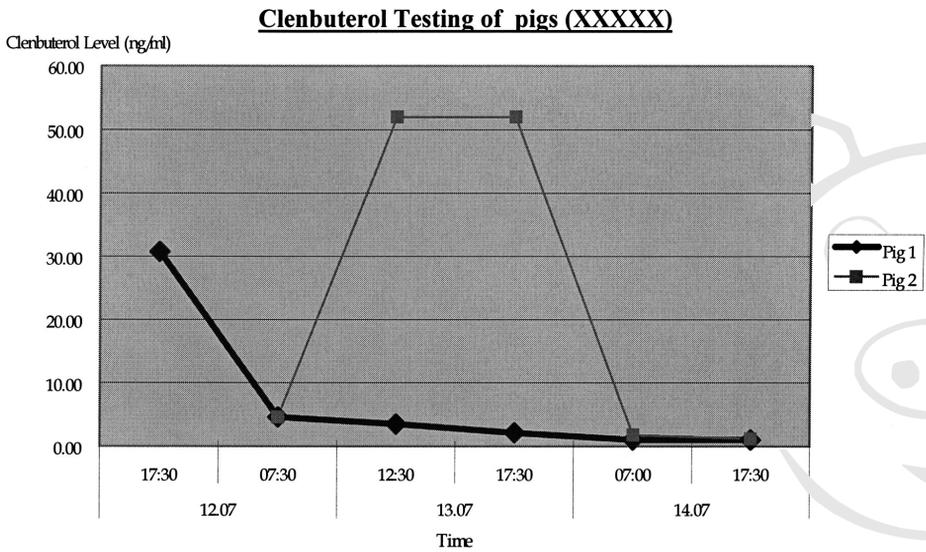
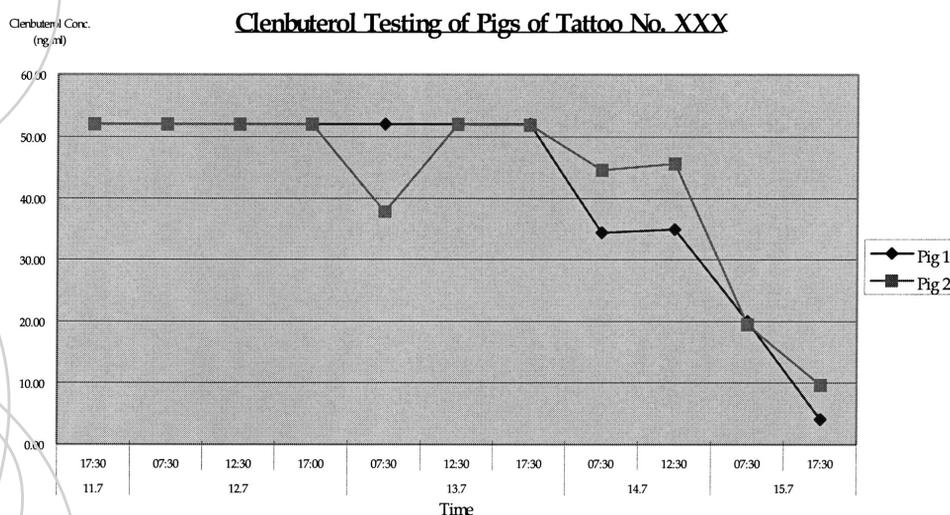


Figure 2d



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**Epidemiological tools (serotyping, resistograms)
for *Salmonella* spp. in pork**

PE 01

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Summary: *Salmonella* spp. isolates from a previous survey were studied with the aim to provide epidemiological data regarding the distribution and spread of the pathogen in pork and pork products in Mexico City, which had been linked to outbreaks. Serotypes and antibiotic resistance profiles of isolates were obtained. Resistance to the most commonly used antibiotics was high and despite the widespread of antibiotic resistant *Salmonella* there was no clear correlation between resistograms and serovars. While serotyping was not useful to establish epidemiological links, cluster analysis (dendograms) provided some information and to suggest that the transfer of resistance vectors might be common. Epidemiologists face an increasing challenge to control infection and trace outbreaks back to primary sources, particularly where foodborne pathogens are endemic in megacities in developing countries where production systems and distribution channels continue to grow in complexity.

Keywords: antibiotic resistance, endemic, dendogram, serovar, processing

Materials and Methods: A total of 154 strains from pork and chorizo samples in Mexico City were isolated and serotyped as described elsewhere (Kuri 1994). Strains from different origin or serotype were assessed for susceptibility to antibiotics using a diffusion test method following Barry and Thornsberry (1985). The Mastring-S System (Mast Laboratories Ltd., Merseyside, UK) was used with three sets of rings (M11, M41 and M26 on AAA 1 & 21 media) and 8 disks each to assess 18 antimicrobials of clinical and epidemiological relevance in relation to Enterobacteriaceae in Mexico and Latin America. Dried plates were inoculated with standardised inoculum and dried. Tempered rings were applied onto the agar and incubated (within 15 min) at 37 °C for 16-18 h. Two perpendicular diameter measurements to the nearest millimetre of the zones of complete inhibition was recorded and analysed to find the minimum inhibitory concentration (MIC) values. Each strain was tested in duplicate and *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* (ATCC 25922, 25923 and 27853) were used as standard controls. To classify the strains according to their susceptibility, USA NCCLS interpretative values (Barry & Thornsberry, 1985) of the areas of inhibition as breakpoints for each drug were used. Else, best discriminations computed from distribution of frequencies of size of inhibition zones of all the strains. Intermediate resistance was not meaningful for most drugs and thus ignored. Only antibiotics useful for discrimination were used to construct the basic resistogram. Average linkage cluster analysis was performed with the antibiotic susceptibilities (mean inhibition diam.) as the degree of relatedness among the strains using a single matching coefficient. Dendograms were plotted and partitions selected. Further analyses included descriptive statistics of the partitions and contingency tables of the relation on the antibiotic resistance grouping and other characteristics of the strains also using Genstat 5, 2.2 (Vax/VMS5).

Results and Discussion: The broad range of serovar distribution showed nearly half of the serotypes present in less than 1/3 of all samples. No single source of contamination or defined pattern was identified. Relationships between antibiotic resistance and serotype were difficult to quantify due to the high number of serovars (Kuri *et al.* 1994). Also, in over a third of the samples containing the pathogen more than one different serovar was isolated. A wide combination of resistance patterns

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was found (Table 1). Statistical analysis showed no significant difference ($p > 0.001$) in the incidence of resistance related to the origin of the isolate. Two partitions selected for further analysis left four ungrouped strains. One with 94 % similarity and 8 main groups and another at 92% similarity and 5 groups

Table 1. Relative proportion resistance to selected drugs of *Salmonella*-resistant isolates from pork (54) and chorizo (36) as percentage of resistant isolates

| Antibiotic | Source (No. of isolates) | | |
|---------------------|-----------------------------|-----------------|---------------|
| | Pork (54) | Chorizo (36) | Total (90) |
| Ampicillin (AP) | 6 | | 3 |
| Cloramphenicol (C) | 12 | 18 | 15 |
| Gentamicin (GM) | 13 | 8 | 11 |
| Kanamycin (K) | 11 | | 7 |
| Nitrofurantoin (NI) | 19 | 22 | 20 |
| Streptomycin (S) | 33 | 36 | 34 |
| Tetracycline (T) | 58 | 52 | 55 |
| None | 26 | 25 | 26 |

Table 2. Average susceptibility to selected agents measured as zones of inhibition (mm) for class strains for partitions (G) formed at 92 % similarity levels from the cluster analysis

| Antibiotic (μg) | Position (Number of strains in group) | | | | |
|---------------------------------|--|-----------|------------|------------|-----------|
| | G1 (26) | G2 (9) | G4 (32) | G6 (10) | G7 (9) |
| AP (25) | 27.7 | 27.1 | 29.0 | 28.6 | 29.0 |
| C | 21.8 | 22.4 | 22.9 | 23.0 | 4.1 |
| Cephalexin | 20.3 | 19.9 | 19.9 | 19.8 | 20.2 |
| GM | 13.5 | 14.2 | 12.8 | 11.9 | 12.6 |
| K | 19.3 | 16.5 | 17.0 | 7.9 | 18.4 |
| NI | 18.4 | 12.7 | 19.2 | 16.1 | 13.9 |
| S (10) | 13.2 | 9.9 | 14.9 | 4.8 | 9.4 |
| T (25) | 9.1 | 18.7 | 21.1 | 5.2 | 0 |
| Trimethoprim | 0 | 0.4 | 0 | 0 | 0 |

(Table 2). Comparison of the means of susceptibility values of every antibiotic for each group were carried out with t-tests. Correlation analysis of the grouping and origin of the strain (product, sample, sampling area in Mexico City), serovar, somatic group, and resistogram was performed as accumulated analysis of deviance for each contingency table (92 % similarity data) and the variables above. Groupings consistently correlated ($p < 0.001$) with the values of antibiotic resistance with the exceptions of 4 drugs. Most clusters contained only two strains and contributed little to the analysis (patterns of occurrence were not significant). One group comprised isolates with different single patterns, sometimes related. The G1 (92% similarity) presented mainly tetracycline single resistance grouping 70 % of *Salm. anatum* and 50 % of *Salm. derby* isolates were obtained from either pork or chorizo samples widespread across all the areas of the city. *Salm. anatum* strains originated either in butchers or street markets. Those 13 strains comprised less than 14 % of those analysed and 10 % of the total isolates. As a representative example, from the 12 isolates of *Salm. bredeney* six strains were procured from the same area, but the rest are equally distributed among 3 zones of the city. The cluster analysis show them distributed between 2 groups, one with seven and other with three isolates, which share the same antibiogram (T S K GM), and originated in different areas from three different supermarket chains; two from the same retailing group sharing their suppliers. Generally, strains from the same sample shared drug susceptibility patterns, and showed with high similarity level in the dendograms, even in cases where they had different serovars.

Conclusions: No defined infection pattern could be established, as no correlation (by analysis of deviance) between the grouping and the origin or serovars of the isolates was observed. No differences were found in *Salmonella* spp. antibiotic susceptibility regarding the source, but links were found by cluster analysis. It is unlikely that the pathogens originated from a single source (e.g. the intestinal contents of the animal) or were introduced from single sources, particularly because multiple serovars are widespread among the herds and perhaps even in single animals multiple *Salmonella* serotypes coinhabit. The problem is complex, and a feasible approach to reducing consumers' risk could be to combine measures to reduce contamination and infection of the products before consumption.

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Association between *Ascaris suum* and *Salmonella enterica* in finisher herds

PE 02

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Summary: A possible association between *Salmonella enterica* and *Ascaris suum* was investigated. *Salmonella* infection was measured by antibodies in meat juice samples taken at slaughter, expressed as an ELISA OD-value. *Ascaris* infection was measured as the presence of white spots in livers detected at slaughter. Data were collected in Denmark in 2001, comprising 128.316 finishers. There was a significant qualitative association between presence of *Salmonella* antibodies and presence of liver spots at herd level. In contrast, no significant quantitative association at herd level was found. Pigs with liver spots had lower antibody levels against *Salmonella* compared with pigs without white spots. However, despite the significant association between *Salmonella* and *Ascaris* at herd level, its causality remains to be determined.

Keywords: Meat juice antibodies, liver white spots, herd level, pig level, relative risk.

Introduction: In Denmark, occurrence of *Salmonella enterica* infections in finisher pigs is monitored by serological surveillance (Mousing et al., 1997). Herds with a high number of seropositive pigs are encouraged to implement intervention measures that will reduce *Salmonella* prevalence in the herd. One of the factors that have been suggested to influence *Salmonella* prevalence in infected herds, and therefore may be considered in an on-farm reduction strategy, is co-infection with parasitic nematodes.

An experimental study has shown a significant interaction between *Salmonella enterica* and the nodular worm of pigs, *Oesophagostomum* spp. In this study, infection with worms enhanced and prolonged excretion of *Salmonella* in inoculated pigs (Steenhard et al., 2002). The aim of the present study was to investigate a possible association between infection with *Salmonella enterica* and the most common parasitic roundworm of pigs in Denmark, *Ascaris suum*, at both herd and pig level.

Materials and Methods: Post mortem data of individual pigs were obtained from the Danish Zoonosis Register and from meat inspection at 4 different abattoirs. *Salmonella* infection was measured by antibodies in meat juice samples taken at slaughter, expressed as an ELISA OD-value. *Ascaris* infection was measured as the presence of white spots in livers at slaughter. Qualitative and quantitative associations were investigated using Mantel-Haenszel (M-H) Chi-square analysis or multivariate regression, with correction for seasonality, abattoir and herd size.

Results and Discussion: Data were collected in 2001, comprising 128.316 finishers. M-H analysis showed a significant association (Relative Risk = 1.13, $p < 0.0001$) between presence of *Salmonella*

antibodies and presence of liver white spots at herd level (see Table 1). However, this analysis does not indicate whether *Salmonella* influences *Ascaris* prevalence or vice versa. In addition, the magnitude of the association is limited although significant, which can be ascribed to the large dataset used. In contrast, there was no association between the number of pigs seropositive for *Salmonella* and the number of pigs with liver white spots. There was no significant interaction of the data with abattoir or season.

Table 1. Distribution of finisher herds with *Salmonella*-positive pigs and *Ascaris*-positive pigs according to slaughterhouse data collected in Denmark in 2001.

| Status | <i>Salmonella</i> positive | <i>Salmonella</i> negative | Total |
|-------------------------|----------------------------|----------------------------|--------|
| <i>Ascaris</i> positive | 3,472 | 6,244 | 9,716 |
| <i>Ascaris</i> negative | 2,408 | 4,442 | 6,850 |
| Total | 5,880 | 10,686 | 16,566 |

Pigs with antibodies against *Salmonella* had a reduced risk of having white spots in their livers (RR = 0.99, $p < 0.05$). However, this Relative Risk is so close to 1 that the association probably has very little biological significance. Pigs with liver white spots had lower antibody levels against *Salmonella* compared with pigs without white spots ($p < 0.0001$), but this association was only borderline significant ($p < 0.10$) when only pigs that sero-reacted against *Salmonella* (OD > 0) were included in the analysis. These results are shown in Table 2. Again, the possibility that the large dataset used enables even minor differences to become significant, without much biological significance, cannot be excluded.

Table 2. Serological response against *Salmonella enterica* in finisher pigs with or without liver white spots caused by *Ascaris suum*.

| Factor | Number of pigs | % positive for <i>Salmonella</i> | Mean corrected OD-value | P-value |
|--------------------|----------------|----------------------------------|-------------------------|---------|
| All pigs: | | | | |
| - no liver spots | 122,438 | 7.1 | 4.3 | <0.0001 |
| - liver spots | 5,878 | 6.0 | 3.8 | |
| Seroreactors only: | | | | |
| - no liver spots | 14,490 | 60.0 | 30.0 | 0.088 |
| - liver spots | 589 | 59.6 | 28.7 | |

Conclusion: There was a significant qualitative association between presence of *Salmonella* antibodies and presence of liver spots at herd level. However, the causality of this relationship could not be determined in this study. Therefore it cannot be excluded that the observed associations can be explained by herd or management factors. The possibility of interaction between *Salmonella enterica* and *Ascaris suum* should be investigated under controlled conditions, e.g. in experimental inoculation studies.

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DNA FINGERPRINTING OF *S. TYPHIMURIUM* FROM A PIG LONGITUDINAL STUDY

PE 04

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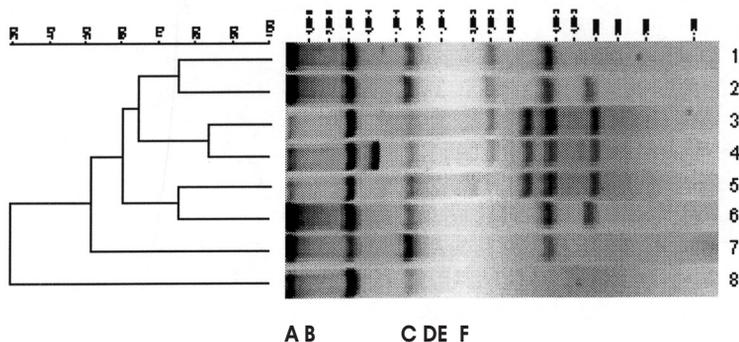
Summary: A 400 sow farrow-to-finish farm was sampled for 6 consecutive years to determine the persistence of *Salmonella* Typhimurium (STM) DT104. Pulsed-field gel electrophoresis, plasmid DNA analysis and antibiotic resistance phenotyping was carried out on selected STM strains, isolated from the farm during the time of the study. Clonal persistence as well and introduction of new clones from external sources were proven to be the main mechanisms by which salmonella infection was maintained in the farm.

Keywords: Pulsed-field gel electrophoresis, plasmid profile, antibiotic resistance, persistence.

Introduction: STM is a common cause of salmonellosis among humans and animals in many countries (Davies, 2001). Phage types DT104 and U302 were the most prevalent types in the UK, in both livestock and humans, in 2001. The aim of this study was to determine the persistence of *Salmonella* Typhimurium DT104 or related phage types in a sow farrow-to-finish pig farm during a 6 year study.

Materials and Methods: Salmonella isolates. 40 STM DT104 or related phage types (DT104, DT104b, DT104c, U302) were selected to represent a wide range of faecal, environmental and wildlife samples from the study farm over time (1996 n = 7, 1997 n = 5, 1998 n = 6, 1999 n = 9, 2000 n = 4, 2001 n = 9). Antibiotic resistance phenotyping: Isolates were screened for susceptibility to a panel of 16 antibiotics on iso-sensitest agar (Oxoid CM471) by a disk diffusion method similar to that previously described (Phillips, 1991). The following disks (Oxoid, Basingstoke, Hampshire) were used: amikacin (10 mg), amoxicillin/clavulanic acid (30 mg), ampicillin (10 mg), apramycin (15 mg), chloramphenicol (10 mg), cefoperazone (30 mg), cefuroxime (30 mg), colistin (25 mg), furazolidone (15 mg), gentamicin (20 mg), nalidixic acid (30 mg), neomycin (10 mg), streptomycin (25 mg), sulphamethoxazole/trimethoprim (25 mg), tetracycline (10 mg) and triple sulphonamide (300 mg). Organisms with a zone diameter of less than 13 mm were classified as resistant. Molecular typing: Plasmid DNA was isolated by the alkaline lysis method and pulsed-field gel electrophoresis (PFGE) with *Xba*I and *Bln*I were performed as described before (Liebana et al., 2002). Fingerprinting data was digitalised and analysed using Gelcompar II (Applied Maths).

Results: All STM isolates from the first 2 years of the study had an identical PFGE type, and 5 plasmids (A, C, D, E, F) were identified amongst them (Figure 1). In 1998 the farm was depopulated, cleaned and disinfected, and was left empty for 6 months. Isolates from samples collected prior to and after cleaning and disinfection (C+D) and also from the replacement animals were studied. The same PFGE type, plasmids and resistance phenotypes were found in isolates from pigs pre depopulation and in isolates from resident mice (droppings) and environment after depopulation and C+D. PFGE and plasmid profiles identified in the first year of the study were still present amongst isolates from 2001, therefore, suggesting clonal persistence. Only, in 2001, did some isolates present variants from the *Xba*I and *Bln*I PFGE types, these were only different in a single band and could represent on-farm diversification of the predominant clone. Plasmids A, C, D, E & F were found on farm isolates throughout the study, possibly indicating that the major plasmid profile groups have evolved from each other by the uptake or loss of one of these plasmids. The fact that isolates belonging to different plasmid profiles have the same *Xba*I and *Bln*I PFGE types supports this hypothesis. Plasmid B was found only in a single isolate from a badger in 2001 that also had different *Xba*I and *Bln*I fingerprints.



PLASMIDS

A B C D E F

Figure 1. Dendrogram generated by the Gel Compar II software showing the relationship of 8 representative plasmid types for 40 *S. Typhimurium* (DT104 and related phage type) isolates. The clustering analysis was performed using the Dice coefficient and unweighted pair group method with arithmetic averages (UPGMA).

The farm was restocked over a period of three months in 1999. A new clone, bearing only the serotype-specific plasmid and distinctive phenotype (resistance to streptomycin and compound sulphonamides), was identified from these replacement animals and remained established in the farm for at least 2 years.

Discussion/Conclusions: We have proven persistence of *S. Typhimurium* clones over a period of 6 years in a pig production system, even after depopulation and C+D. The isolates showed the same genomic PFGE type (*Xba*I and *Bln*I). The plasmid profiles for these isolates were made up of one or more of only 6 plasmids, possibly indicating that the major profile groups have evolved from each other by the uptake and loss of one of these plasmids. We presented definitive proof that wildlife act as a reservoir of *S. Typhimurium* in the environment, and also that new breeding stock is often an additional source of new clones.

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Longitudinal study of *Salmonella enterica* serovar Typhimurium infection in three Danish farrow-to-finish swineherds

PE 05

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Summary: A longitudinal study on *Salmonella enterica* was carried out in 3 Danish farrow-to-finish swineherds in 2001. Litters from each herd were divided into 2 cohorts of 30 pigs each (180 pigs in total). Individual pigs were examined for bacteriology and serology monthly from weaning to slaughter. At weaning, individual sows were also examined for bacteriology and serology. In total, 88 pigs were found to be shedding on ≥ 1 occasion. Only *Salmonella enterica* serovar Typhimurium was detected. The culture-prevalence peaked in the nursery, and subsequently declined to undetectable levels before slaughter. The sero-prevalence peaked approximately 60 days after peak culture-prevalence. *Salmonella* was detected in individual fecal samples at least once in 53% of the pigs, while 62% were sero-positive more than once. Only 3.7% of all pigs were found to be culture-positive on ≥ 1 occasion. The average shedding time was estimated to have been 18 days.

Keywords: transmission, dynamics, duration of infection, animal health, surveillance

Introduction: To ensure food safety, continuing efforts are needed to reduce the occurrence of *Salmonella* in pork. In order to achieve this, information about the dynamics of *Salmonella* infections in swineherds over time (e.g. duration of infection and disease transmission patterns) is important. However, so far only limited information about this aspect of *Salmonella* infection has been available. Therefore, a longitudinal study of the infection dynamics of *Salmonella enterica* was carried out in three Danish farrow-to-finish swineherds. The aim was to elucidate the complex nature of sub-clinical *Salmonella* infections at herd and pig level. Particularly, the association between bacteriological shedding and serological response in cohorts of pigs from weaning to slaughter was investigated.

Materials and Methods: Three farrow-to-finish swineherds with moderate to high levels of *Salmonella* were selected for the study, based on serological and bacteriological data from the national *Salmonella* surveillance program in Denmark. To ensure that *Salmonella* was present in all age groups, pooled pen fecal samples were collected prior to the start of the study.

To account for variations in *Salmonella* shedding over time, litters from each herd were divided into 2 cohorts that were raised with approximately 1-month intervals. Each cohort consisted of 30 pigs each, yielding a total of 180 pigs. Individual pigs were examined for bacteriology and serology monthly from weaning to slaughter. At weaning, individual sows were also examined for bacteriology and serology. Finally, cecal-content samples, ileo-cecal lymph nodes, and carcass swabs were obtained from 131 pigs at slaughter.

Serological examinations were performed using the Danish Mix-ELISA with a cut-off level of OD $>$ 20. Feces were cultured using standard microbiological methods, including non-selective pre-enrichment, selective enrichment and serotyping.

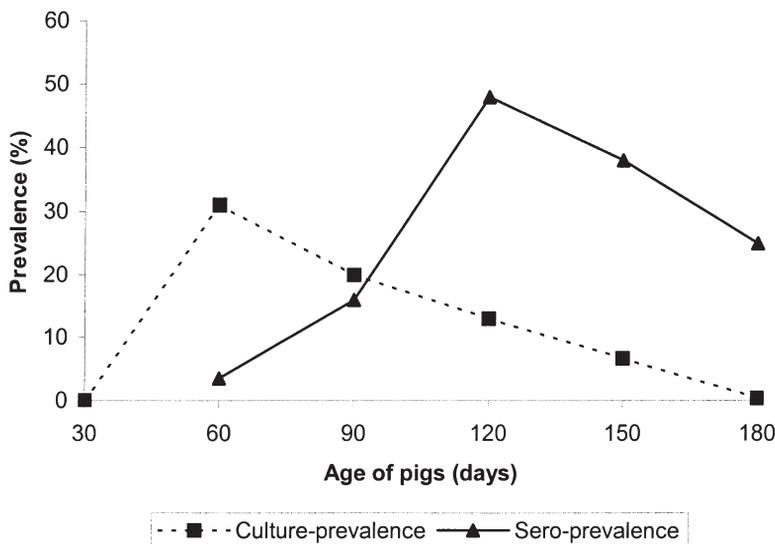


Figure 1: *Salmonella* prevalence in blood and feces samples across three Danish farrow-to-finish swineherds according to pig age, 2001.

Results: In total, 88 pigs were found to be shedding on one or more occasions. On all three farms, only *Salmonella* Typhimurium was isolated during the study period. At weaning, no sows or piglets were found to be shedding, but serological reaction was detected in 11 sows. The culture-prevalence peaked in the nursery, and subsequently declined to undetectable levels before slaughter. The sero-prevalence peaked approximately 60 days after peak culture-prevalence (Fig. 1). *Salmonella* was detected in individual fecal samples at least once in 53% of the pigs, while 62% were sero-positive more than once. Only 3.7% of all pigs were found to be culture-positive on more than one occasion. Marked differences in culture- and sero-prevalences between cohorts and within herds were observed. Piglets from sero-reacting sows had a significantly ($P < 0.05$) lower probability of being shedders in the nursery. Under the assumption that shedding had started 0.5 week before the first positive fecal sample, and lasted until at least 0.5 week after the last positive fecal sample, the average shedding time was estimated to have been 18 days.

Discussion: There are several explanations for time of onset and course of *Salmonella* shedding: (a) piglets became infected in the farrowing unit, without shedding; (b) protecting factors in the farrowing unit had a reducing effect on *Salmonella* shedding by piglets; (c) weaning stress triggered shedding in the nursery; (d) residual infection was present in the nursery despite cleaning and disinfection; (e) contaminated tools, boots, etc. were present in the nursery. The last two explanations seem most likely, as it has been shown that strategic movement of pigs at weaning can eliminate infection (Dahl et al., 1997).

Infection spread to most of the population, but less than 4% of the pigs were found to be culture-positive on more than one sampling occasion (fecal samples were taken at 4-week intervals), indicating an average duration of infection of 2-3 weeks. Furthermore, during the finisher stage *Salmonella* shedding decreased with age (Figure 1). This implies, that the *Salmonella* prevalence among finishers from a herd infected with *Salmonella* can be low or negligible at the time of slaughter, reducing the risk for human health.

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Determination of *Toxoplasma gondii* Antibody Prevalence in Midwest Market Swine

PE 06

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Keywords: food safety, ELISA, meat juice, zoonosis, surveillance

Summary: Pork has been identified as one of the food source(s) for human exposure to *Toxoplasma gondii*. This project was designed to determine the current prevalence of *Toxoplasma gondii* antibodies in the Midwestern USA market swine population. Test samples were selected, using random numbers generated from the Excel database, from approximately 2,500 daily meat juice samples submitted for Aujeszky's Disease from eight Iowa abattoirs. Producer identification and lot size were recorded for each lot. Two hundred fifty samples were selected for 12 consecutive weeks – total of 15,014 samples. The presence of antibodies was determined using ELISA test kits by Safepath Laboratories. The prevalence for all samples was 0.75 % with a higher prevalence found in lots of 20 - 40 compared to 150 - 190 head. Additional on-farm evaluations of exposure risk factors are required to determine an association between sero-prevalence and lot size and to develop suitable prevention strategies.

Introduction: *Toxoplasma gondii*, an intracellular protozoan of cats, is an important public health concern. Human infection may occur by two primary routes: 1) direct contact with soils or foods contaminated with cat feces; or 2) consumption of undercooked meat from an animal previously infected with *Toxoplasma gondii*. *Toxoplasma gondii* infections in rodents, swine, sheep, cattle and perhaps poultry result from exposure to cats feces or contaminated environments. The infectious stage (bradyzoites) may survive in muscle and brain tissues for the animal's life. Pork has been identified as one food source for this parasite.

Production practices may influence the prevalence of infected animals. Infected cats shed large numbers of oocysts in feces for approximately 10-20 d. Once shed, oocysts are environmentally stable. Therefore the environment remains contaminated for extended periods after cats are removed or become on-infectious carriers. Few oocysts are required to infect swine. Removal of direct contact with cats, and cat feces or a contaminated environment minimizes potential transmission. Keeping cats from contact with feed or soils near production reduces exposures. It is possible that soil transferred on boots may be sufficient for infection of swine. Rodents (mice predominately) may be a source of infection for cats and for swine. Prior prevalence studies have indicated infection levels of 20 - 43 % in breeding animals and 0.14 - 5 % in finishers depending on the sample populations, locations of operations and sampling period (R. Gamble, personal communication, 2001). In many cases outside production and exposures to cats or cat feces were identified as risk factors (Leighty, J.C., 1990; Weigel et. al., 1995). As pork production has become more confined a question has been raised about the prevalence of *Toxoplasma* infections.

Objectives: To evaluate the presence of *Toxoplasma* antibodies as measured by a meat juice-based ELISA detection procedure in Midwestern market swine.

Materials and methods: Meat juice samples were selected from a population collected for the PRV market swine surveillance project. This market surveillance collected four (4) meat samples from each lot of swine at eight (8) high volume Iowa abattoirs. Approximately 600 lots were collected daily and submitted to the Iowa State University meat juice processing laboratory of processing and

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PRV antibody analysis. Each sample was maintained with a unique identifier that enabled trace back to the submitting producer.

Samples for *Toxoplasma* antibody detection were selected randomly from the daily submissions during the spring of 2002. Two hundred fifty (250) samples were selected each day for 12 consecutive weeks (60 sample d) – total of 15,014 samples. As part of the random sampling algorithm only one sample from a producer was selected each day, even if multiple lots were submitted to single or multiple plants from that producer. Producer identification and lots size submitted were recorded. The presence of *Toxoplasma* antibodies was determined using the ELISA test kits supplied by Safepath Laboratories (Carlsbad, Calif. USA). Samples were diluted 1:10, according to manufacturer's recommendations for meat juice prior to testing. Results were reported as positive using a > 0.20 O.D. breakpoint.

Results/Discussion: A total of 15,014 samples were collected from 3690 producers from 16 Midwestern states. Mean lot size was 92 head, but a clear bi-modal distribution existed. Approximately 60 % of the lots were from 20 - 50 head/lot and 30 % from 160 - 200 head/lot, with the remainder arrayed between these lot sizes. One hundred thirteen (113) samples were positive for an individual animal prevalence of 0.75 %. Eighty-eight (88, 2.3%) producers were identified with a single positive sample. Sixteen (16) producers, including four identified as order buyers/buying stations, had two or more positive samples. Comparisons of the lot size/positive values interaction indicated that 86/113 positives were found in lot sizes < 50 head, and were 2 times more likely to be serologically positive than larger lots (OR= 2.1, 95% CL, 1.4-3.5). Only 11/113 were identified in lots > 100 head. All producers with multiple positive results were from the smaller lots.

Conclusions: The observed level of 0.75 % is consistent with a continued prevalence reduction observed in earlier studies and observations (0.80%) in the 2000 NAHMS survey. The association between positive samples in the smaller lot sizes may indicate a higher risk associated with more extensive production practices, however other confounding factors may be involved. These results are consistent with earlier evaluations of risk factors at the production level. These risk factor issues require further examination as production-based *Toxoplasma* control programs are developed. This project also demonstrates the value of meat juice technology in market swine surveillance of zoonotic disease.

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THE USE OF A HACCP-BASED CONTROL SYSTEM IN CLOSED PIG HERDS

PE 07

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Summary: To fulfil the consumer demands about food safety, quality control systems are necessary in the entire food production chain, including pig production. One of the tools to control quality is a HACCP-system, based on control points. The aim of present study was to define control points in 12 closed pig herds based on slaughterhouse data. Herd data, obtained by questionnaires, comprised data about housing and ventilation, management, feeding, hygiene, prevention of diseases and transport to the slaughterhouse. Outcome variables for the univariate analysis were the percentage of animals with lung lesions, pleuritis, dermatitis scores, white spots on the liver and the *Salmonella* prevalence. The results of this study should be interpreted with caution, since the analysis was carried out on no more than 12 herds and because no multivariate analysis was done. However, it is clear that slaughterhouse data are essential for the farmer to optimize the production of healthy and safe pigs.

Introduction: Quality can be ensured by the use of quality control systems such as a HACCP (Hazard Analysis of Critical Control Points)-based system, based on risk factors or critical control points (Anonymous, 1991). The use of such a system in the pig herds has recently been introduced. The aim of present study was to find the risk indicators for lung lesions, white spots on the liver, *Sarcoptes scabiei* and *Salmonella* by use of slaughterhouse data.

Materials and Methods: Herd data were collected by means of a questionnaire on 12 farrow-to-finish herds belonging to one slaughterhouse co-operation. The questionnaire comprised information about management, housing and ventilation, feeding, hygiene and biosecurity, prevention of diseases and transport to the slaughterhouse. Following slaughterhouse data were obtained from 10 consecutive slaughterhouse deliveries per herd: the percentage of animals with white spots on the liver, with *Sarcoptes scabiei* dermatitis, with lung lesions and with pleuritis. The percentage of *Salmonella*-infected animals was determined in a previous field study and was based on bacteriological isolation in lymph nodes. Data were analysed using a univariate analysis at a level of significance 0.2 (SPSS 11.0).

Results: The percentage of animals with white spots on the liver, with *Sarcoptes scabiei* dermatitis, with lung lesions and with pleuritis was 12.3%, 0.6%, 8.5% and 6.5%, respectively. The average number of *Salmonella*-infected animals was 38.4%. The most important risk indicator for white spots was the management system, with an all-in/all-out system protective compared to a continuous system ($p=0.13$). *Scarcoptes scabiei* could be prevented by the systematic use of antiparasititcal drugs ($p=0.10$). Also quality label organisations can impose measures to reduce the prevalence of *Scarcoptes scabiei* ($p=0.00$). Important factors to prevent lung lesions are an adequate ventilation system ($p=0.13$), the implementation of a quarantine period for at least 4 weeks ($p=0.16$), the use of an all-in/all-out production system ($p=0.04$), a stand-empty period for minimum 3 days ($p=0.05$), the preventive use of antibiotics ($p=0.20$) and vaccination of boars and sows against PRRSV ($p=0.03$). The percentage of animals with pleuritis could be reduced by the use of a stand-empty period for minimum 3 days in the finishing unit ($p=0.19$), the correct use of ventilation systems ($p=0.13$), the eradication of rodents ($p=0.19$), the use of an all-in/all-out system ($p=0.08$) and vaccination of boars and sows against PRRSV ($p=0.03$) and Influenza ($p=0.18$). Important protective factors associated with a lower *Salmonella*-prevalence are the purchase of boars ($p=0.02$), the feeding of meal ($p=0.08$), the use of all-in/all-out

($p=0.13$), an adequate rodent eradication program ($p=0.01$) and the with-holding of feed for maximum 18 hours before transport to the slaughterhouse ($p=0.13$).

Discussion: The application of a herd-specific HACCP-plan on pig herds can be a useful tool to guarantee product quality and safety and can be part of an Integrated Quality Control System (Noordhuizen and Frankena, 1999). In the present study, possible risk indicators for several diseases were investigated. This risk analysis can be the basis for the determination of the critical control points. It is important for the farmer to obtain feed-back from the slaughterhouse to be informed about the disease status of his animals. Several diseases, like *Salmonella*, occur subclinically in pigs, but can cause human disease. Other infectious agents, like *Mycoplasma*, *Actinobacillus pleuropneumoniae*, *Ascaris suum*, or *Sarcoptes scabiei*, do not manifest clinical symptoms or visual lesions, but can be the cause of a reduced feed consumption and subsequently retarded growth. The risk factors were the result of a univariate analysis with significance at the 0.2 level. This means that the results should be interpreted with caution and no definitive conclusions can be drawn. Another limiting factor in the study is the low number of herds, due to by practical and budget considerations.

The strict use of a HACCP-system on pig herds however is a utopia. It is impossible to control all risk factors, since the product is a living animal which is subject to a lot of variable factors. However, a HACCP-based system can be useful to reduce the hazards associated with the consumption of meat.

Conclusions: Pig farmers can apply a HACCP-based quality system to ensure the delivery of healthy and safe pigs to the slaughterhouse. It is possible to define risk indicators and consequently control points by feedback of slaughterhouse data.

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PE 09

USDA Multi-Agency Project: Collaboration in Animal Health, Food Safety & Epidemiology (CAHFSE)

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Summary: Despite producer interventions, on-going research and continued surveillance, food borne outbreaks continue and multiple antimicrobial resistant bacteria have emerged. A multi-agency APublic Health Action Plan to Combat Antimicrobial Resistance@ was developed to address these concerns and one USDA response was the development of the Collaboration in Animal Health, Food Safety and Epidemiology (CAHFSE), a partnership among the Agriculture Research Service (ARS), Animal and Plant Health Inspection Service (APHIS), and Food Safety Inspection Service (FSIS). The objective of CAHFSE is to implement and expand a surveillance system patterned after the APHIS National Animal Health Monitoring System (NAHMS) which focuses on animal health and food safety. Swine is the first commodity in CAHFSE. To date, fecal samples from 8 farms have been collected and processed for culture of *Salmonella*, *Campylobacter*, *Enterococci* and *E. coli*. Preliminary results indicate that all four bacteria have been recovered from a number of operations and are currently being characterized.

Keywords: Swine, Pork, USDA, Food safety

Introduction: Food animals are an important source of bacteria causing illness in humans. *Campylobacter* and non-typhoidal *Salmonella* spp. account for 2.4 and 1.4 million cases, respectively, of acute bacterial gastroenteritis in humans annually in the United States (Mead et al. 1999). Because of these outbreaks and emergence of antimicrobial resistance among food borne bacteria, the scientific community and public health officials have examined antimicrobial use in food animal production (Levy, 1992; FDA, 1998; WHO, 1997). Also, a multi-agency APublic Health Action Plan to Combat Antimicrobial ResistanceA was developed to address these concerns (CDC 2003). USDA responded by developing the Collaboration in Animal Health, Food Safety and Epidemiology (CAHFSE), a partnership among the Agricultural Research Service (ARS), Animal and Plant Health Inspection Service (APHIS), and Food Safety Inspection Service (FSIS). The objectives of CAHFSE are: 1) to enhance understanding of pathogens that pose a food-safety risk by tracking these pathogens on the farm and to the plant and 2) to routinely monitor critical diseases in food-animal production. Factors associated with the development of antimicrobial resistance will also be addressed. Swine is the first commodity in CAHFSE.

Materials and Methods: Twelve swine farms in each of four states (Iowa, Minnesota, North Carolina and Texas), were selected based on a quota sampling scheme approximated from distribution in the National Animal Health Monitoring System=s (NAHMS) Swine 2000 study. Participation is voluntary. Selection criteria included production types (indoor farrow-to-finish, outdoor farrow-to-finish, indoor finish only, and outdoor finish only) and swine density in respective counties within each state.

Samples are collected quarterly and during each site visit a questionnaire regarding animal inventory, animal health, and antimicrobial use is conducted. Blood is collected from 15 market pigs which are 6-8 wks, 11-13 wks, 16-18 wks, and > 22 wks old for *Lawsonia intracellularis* serological testing. In addition, 40 pen floor fecal samples are collected from pigs at least 22 wks old for culture, isolation, and characterization of *Salmonella*, *Campylobacter*, *Enterococci*, and *E. coli*. All *Salmonella*, *Enterococcus* and *E. coli* isolates are tested for susceptibility to antimicrobials using a semiautomated broth microdilution system (Sensititre™, Trek Diagnostics, Westlake, OH) and *Campylobacter* are tested using the E-Test (AB Biodisk, Piscataway, NJ). The antimicrobials used in evaluating the respective bacterial species are as described for the National Antimicrobial Resistance Monitoring System (NARMS). Selected isolates will be characterized by molecular techniques to determine relatedness.

Sample collection at slaughter (carcass swabs, lymph nodes, and ground product) will be implemented in 2004. Plant samples will be cultured and characterized similarly to on-farm isolates. Slaughter findings will be linked with on-farm and in-plant questionnaire and laboratory data to determine risk factors.

Results: To date, fecal samples from 8 farms have been collected and processed for culture of *Salmonella*, *Campylobacter*, *Enterococci* and *E. coli*. Preliminary results indicate that all four bacteria have been recovered from a number of operations and are currently being characterized.

Discussion: The NAHMS Swine 2000 study (APHIS 2002) indicated antimicrobials were fed to grower/finisher pigs on 88.5% of swine farms, which represent 95.9% of grower/finisher pigs in the USA. Thus, antimicrobial use and related issues are a major concern to the pork industry. Little information is available over time regarding production uses of antimicrobials on the development and persistence of antimicrobial resistance among enteric bacterial species. CAHFSE will provide on-farm and in-plant trends in prevalence, antimicrobial susceptibility, and genetic relatedness of enteric bacterial species. Isolate characterization trends will be correlated to management practices to help identify risk factors associated with antimicrobial resistant bacterial species in food animals and their products.

CAHFSE will also provide an epidemiological description of *Lawsonia intracellularis* associated ileitis in multiple age groups of weaned market pigs. Prevalence of *Lawsonia intracellularis* and resulting morbidity and mortality rates shall be correlated with management practices and operation facilities.

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PE 10 REPEATED OBSERVATIONS ON THE SALMONELLA CULTURE STATUS OF MIDWEST U.S. HERDS

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Summary: Mesenteric lymph nodes were collected from pigs from 115 Midwest U.S. swine herds at slaughter on two occasions separated by 6-9 months. These herds were sampled up to three additional times during a three-year period, with 30 herds sampled five times. Thirty pigs were sampled at each collection. Herds were categorized positive if one or more samples revealed *Salmonella* spp. While culture status at collection one was associated with the second sampling collection ($p < 0.01$), the association was only moderate in strength (OR = 2.6). Herds with three consecutive positive tests (9 of 38) were all positive on sample four. Prevalence estimates were weakly or not correlated between samplings. In conclusion, *Salmonella* culture status of these swine herds was weakly predictive of future culture results. Accurate description of *Salmonella* status based on bacterial culture appears to require repeated or ongoing testing.

Keywords: swine, predictability, *Salmonella* shedding, slaughter

Introduction: *Salmonella* carriage among pigs at slaughter poses a potential public health threat, if hygiene practices at the time of slaughter may fail to prevent the transmission of the bacteria through the food chain. Although pigs have been shown to shed *Salmonella* on the farm and after transport and time in lairage (Hurd, et al.) the bacteria can be brought forward from the farm. Therefore, it may be useful to categorize herds by *Salmonella* status prior to shipment. For this to be most effective, the *Salmonella* status or prevalence should be predictable over time. However, little has

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been reported on the on-farm shedding or culture positive status of herds at slaughter over extended periods. We designed this study to assess the repeatability of *Salmonella* culture status of commercial pig herds over a three year period.

Methods and Materials: Herds were solicited to participate in this study as a follow-up to an assessment of *Salmonella* prevalence among 141 Midwest herds marketing to one of two major slaughter plants. (Damman, et al.) All herds selected for study were eligible for up to five follow-up observations over a three-year period. Sampling was delayed a minimum of six months. Samples were then collected on the next available marketing, laboratory resources permitting. Where laboratory resources were overcommitted, the farm was sampled again at the next marketing to the plant.

Ten-gram samples of caudal mesenteric lymph nodes were aseptically collected after evisceration by dissection of overlying mesentery. Conventional bacterial culture methods were used to isolate *Salmonella*, using a slight modification of a previously described procedure. (Fedorka-Cray, et al.) For the first sampling collected from each farm five samples were pooled, combining two grams from each pig. Samples were blended and incubated in tetrathionate broth at 37°C for 48 hrs. One ml of this broth was transferred to R-10 broth and incubated 24 hrs at 37°C. XLT-4 plates were streaked for isolation, followed by culture on Brilliant Green agar, and finally suspect colonies were tested for agglutination with polyvalent anti-*Salmonella* sera. From positive pools, retained frozen tissue was cultured individually using two-gram samples in 20 ml of tetrathionate. This same individual procedure was used for collections two through five, with no pooling of samples.

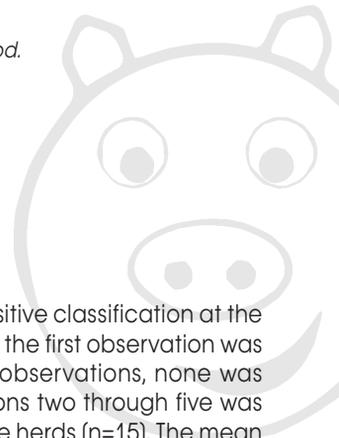
Prevalence estimates were compared between collections using pair wise Spearman's Rank correlation. Herds were also categorized positive / negative if one or more sample was positive. Categorical tests of association were pair wise agreement, kappa statistic, and the examination of repeated consistent test results as a predictor of the subsequent test.

Results: The number of herds sampled declined over the period as farms marketed to other plants, or stopped delivering pigs for other reasons (Table 1).

Table 1. The number of swine herds sampled for *Salmonella* spp. over a 3-year period.

| Collection | n. herds |
|------------|----------|
| 1 | 144 |
| 2 | 116 |
| 3 | 56 |
| 4 | 40 |
| 5 | 34 |

Positive classification at the first collection was positively associated with positive classification at the second observation, kappa = 0.23, $p < 0.01$, OR = 2.6. However, classification at the first observation was not associated with the fifth observation ($p > 0.2$). Of thirty herds with five observations, none was negative on all five collections. The proportion of herds positive on collections two through five was similar between collection one positive herds ($n = 15$) and stage one negative herds ($n = 15$). The mean proportion of positive herd tests in the second through the fifth collections was 37% among herds positive for collection one, and was 42% for herds negative on collection one. Of herds with four sequential observations (collections 1-4), the nine that were positive on all of the first three sequential samples were all positive on collection four. In contrast, on collection four, 18 of 29 herds were positive among those that were negative on one or more of the first three collections. Among the pair-wise comparisons, the Spearman Rank coefficients for paired comparisons which had $p < 0.05$ and the values of the coefficients were collections 1 and 2 (0.32), 1 and 4 (-0.52), 1 and 5 (0.58) and 2 and 5 (0.61).



Discussion: The odds ratio linking collection one status vs. collection two status of 2.6 suggests that herds positive for collection one were more than two times more likely to be positive on the subsequent shipment. However, this association was demonstrated when examining other pairs of results. The limited correlations between prevalence of samples one and two suggests that there is some repeatability. However, this relationship was moderate in strength, and also was not seen in other pairings.

Since these samples were collected at the slaughter plant, it is possible that *Salmonella* isolates originated from trucks or from the lairage at the plant. If this were the case, associations between sequential collections would be weakened, since the exposure after the farm gate might not be associated with the shedding status of the farm. Another study of these same farms, however, has demonstrated a strong correlation between the *Salmonella* spp. prevalence among fecal samples collected immediately before shipment and prevalence detected in caudal mesenteric lymph nodes from the same pigs at slaughter. (Kim, et al.) Thus, it seems probable that the poor correlations and associations observed between sequential samples reflects changes on the farm in addition to variation caused by bacteria acquired after leaving the farm.

We conclude that single time bacterial culture of mesenteric lymph nodes at slaughter is relatively poor predictor of subsequent test results of a farm. Accurate description of *Salmonella* bacterial culture status requires repeated or ongoing sampling, particularly at the farm level.

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PE 11

Salmonella infection in a multiple-site swine production system in Brazil

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Summary: A longitudinal study was conducted in a multiple-site swine production system. Individually identified piglets were sampled for *Salmonella* fecal excretion and serology. Furthermore, intestinal content, mesenteric lymph nodes and blood samples were taken from these animals at slaughter. In addition, feed samples were taken throughout the study period. Piglets were fecal-culture and serology negative until the nursery phase, but became *Salmonella* positive in the early finishing phase. On this sampling day, 28.6% of finishers were seropositive and 75% were shedding *Salmonella* in feces. At slaughter, the seroprevalence (76.9%) was higher than in the early finishing, but *Salmonella* was isolated from intestinal content or mesenteric lymph nodes in only 19.2% of the sampled pigs. *Salmonella* was isolated from three out of 26 feed samples, being all positive samples collected during the finishing period. In spite of being isolated from different system sites, 89.56% of all *Salmonella* strains belong to serovar Typhimurium

Keywords: longitudinal study, bacteriological, serological

Introduction: Pigs can become infected with *Salmonella* at the farm, during transportation, at the lairage or at slaughter (Swanenburg et al., 2001). Previous studies conducted in southern Brazil demonstrated that *Salmonella* could be isolated from healthy slaughtered pigs. Furthermore, the prevalence of carrier animals at slaughter contributed to the contamination of carcasses and of pork products (Castagna et al., 2001). On the other hand, control programs to reduce *Salmonella* in pork should include monitoring at farm level (Funk et al., 2001). In the present study a cohort of pigs was followed from farrowing to slaughter, in order to demonstrate when the *Salmonella* infection occurred.

Material and Methods: Nineteen sows from a farrowing unit, that presented *Salmonella* positive animals in a previous bacteriological evaluation, were randomly chosen and included in the study. From each sow blood and feces were taken on day 100 of gestation (n=19) and on day 15 of lactation. During this visit, 99 piglets from their litters were individually identified and also included in the study. Subsequently, all pigs were sampled for blood and feces on day 38 and a sub-set of them (n=56) on day 59 and on day 80. Furthermore, samples of feed and environmental swabs were collected at the farm, in the transportation truck and at the lairage. At slaughter, intestinal content, mesenteric lymph nodes and blood samples were taken from 26 pigs of the cohort. The isolation of *Salmonella* followed the previously described protocol (Michael et al., 1999). Serum samples were tested through an ELISA test using *Salmonella* Typhimurium LPS antigens. The cutoff was calculated based on the optic density mean of a negative population with four standards deviations added (Kich, 2003).

Results: 94.7% (18/19) of sows sampled during gestation were seropositives, but none was excreting *Salmonella* in feces on the sampling day. On day 15 of lactation seroprevalence of the group reduced to 66.7% (10/15), but two sows were shedding *Salmonella*. Piglets were negative on serology and *Salmonella* fecal isolation until the nursery phase. However, in the finishing, 28.6% (16/56) seroconverted and 75% (42/56) were shedding *Salmonella* in feces. At slaughter the seroprevalence was 76.9% (20/26), and *Salmonella* was isolated from intestinal content or mesenteric lymph nodes in 19.2% (5/26) of the sampled pigs (Table 1). Contamination of the environment was found at the finishing site only after animals were housed, but the lairage was already positive before pigs entered. It was possible to isolate *Salmonella* from 3/26 samples of feed, and all positive samples were collected during the finishing phase. Serovar Typhimurium was isolated from all positive animals during the finishing, but from two animals serovar Senftenberg was isolated concomitantly. At slaughter, serovars Typhimurium and Senftenberg were found in three and two animals, respectively. All isolates of feed samples belonged to serovar Senftenberg. Strains isolated from the environment belonged to serovars Typhimurium and Panama.

Discussion: As previously related (Kranker et al., 2002), the shedding and seroprevalence of *Salmonella* presented marked variation. In contrast to previous studies (Berends et al., 1996.), where the reproduction units were pointed out as responsible for up to 10% of the *Salmonella* contamination of herds, in the present study pigs became infected only during the finishing. In spite of the decrease in the *Salmonella* shedding observed at slaughter, these animals remain a hazard of contamination for negative pigs during the lairage, as previously demonstrated (Van der Gaag et al., 2003). The isolation of serovar Senftenberg from animals at finishing and at slaughter as well as from feed samples indicated feed as the probable contamination source of this herd.

Conclusions: These results indicate the importance of the finishing period for the diffusion of the *Salmonella* infection in this production system and demonstrate the possible relation with the consumption of contaminated feed.

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Table 1. Distribution of pigs according to bacteriological and serological results at the finishing phase (n=56) and at slaughter (n=26).

| | Finishing | | Slaughter | |
|----------------|--------------------|--------------------|--------------------|--------------------|
| | Isolation Positive | Isolation Negative | Isolation Positive | Isolation Negative |
| ELISA Positive | 13 | 3 | 3 | 17 |
| ELISA Negative | 29 | 11 | 2 | 4 |

PH 01

Prevalence and number of *Salmonella* in retail pork sausages

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Summary: The aim of this study was to assess the prevalence of *Salmonella* in Irish pork sausage at retail level. Samples, comprising branded prepacked sausages, loose sausages from supermarket meat counters and butcher shops, were collected from selected retail sites in four cities from October to December 2001 and from June to August 2002. A 3-tube Most Probable Number (MPN) method was used to enumerate *Salmonella* in a selected number of samples, which were positive by enrichment. *Salmonella* serotypes were detected in 4.4% and 1.7% of samples at each of the respective sampling periods; a level similar to those reported in other U.S. and U.K. studies. Limited results available on enumeration suggest that contamination rates were low. This study revealed that *Salmonella* are present in a proportion of Irish sausages and further risk analysis work is necessary in order to quantify

the risk posed to public health. Keywords: control programme, enumeration, serology, bacteriology, food safety.

Introduction: In Ireland, the crude incidence rate of reported human cases of salmonellosis, over 60% of which were due to *Salmonella enterica* serotype Typhimurium, rose in the 1990s to peak in 1998. Worldwide, the principal sources of *Salmonella* infection in humans are poultry, poultry products and eggs. However in Ireland control of *Salmonella* infection in the poultry industry has been highly effective as evidenced by the decreasing rate of isolation of *Salmonella* Typhimurium or *Salmonella* Enteritidis in chickens. Nevertheless, these serotypes represent the predominant serotypes associated with human infection in Ireland (FSAI, 2000). This gives rise to concern that there may be significant sources of *Salmonella* infection in Ireland other than poultry. A National *Salmonella* control programme in the pork industry was enacted in Ireland in August 2002. This study was undertaken as part of a larger project investigating the role of pork as a source of human salmonellosis in Ireland. The objectives of this study were 1) to determine the prevalence of *Salmonella* in pork sausage and 2) to ascertain contamination levels of *Salmonella* in sausage as part of the assessment of the role of pork as a source of human salmonellosis in Ireland.

Materials and Methods:

Retail Market survey Half-pound (200g) samples of fresh pork sausage were collected at weekly intervals over 8-week periods in 4 cities; Dublin, Cork, Galway and Athlone. Four hundred and fifty five samples were purchased at supermarkets (branded prepacked and loose-type) and butcher shops during the months of October to December 2001, while 466 samples were purchased during the months of June to August 2002. A total of eight supermarkets (different retail chains) and up to forty-eight butcher shops were sampled in each city. Samples were stored in a chilled container during transport and kept at 4AC prior to examination within twenty-four hours of purchase.

Microbiological analysis *Salmonella* isolation procedures were performed on 25g of each sausage sample according to BS EN 12824; 1998. Briefly, samples were pre-enriched in 225ml BPW and incubated for 16-24h at 37AC, followed by selective enrichment for 18h-24h in both Rappaport-Vassiliadis broth and selenite-cysteine broth at 41.5AC and 37AC respectively. Samples were plated onto mannitol lysine crystal violet brilliant green agar (MLCB) and brilliant green agar (BG) after both 24h and 48h of selective enrichment. Up to 5 suspect colonies per plate were identified by subculture onto MacConkey agar and inoculation of triple sugar iron agar slopes followed by serotyping.

MPN (Most Probable Number) Analysis An estimation of the number of *Salmonella* spp. in a selected number of samples was determined using a modified 3-tube MPN method (Dufrenne et al. 2001). These samples had been stored at -20AC for between 8 - 20 weeks.

Isolation of *Salmonella* serotypes from 3x50ml, 3x5ml and 3x0.5ml aliquots of homogenised sample in BPW was performed as described above. After confirmation, the number of *Salmonella* present in each sample was calculated using the MPN table of de Man (De Man 1983).

Statistical Analysis *Salmonella* prevalence was reported as the percentage of samples that tested positive. Differences in prevalence between product types, cities and time were compared using the chi square option of the frequency procedure of the Statistical Analysis Systems (SAS) and the Fischer's Exact Test.

Results: A summary of the *Salmonella* isolation rates is shown in Figure 1. During Part 1 of the survey (Oct-Dec 2001) 20/455 (4.4%) samples were positive for *Salmonella*. A total of 466 samples were tested in Part 2 of the study (June - Aug 2002) and 7 of these (1.5%) were positive for *Salmonella*. There was no significant difference in *Salmonella* prevalence between the four cities, from which samples were collected. However, there was a trend for butcher shop samples to be contaminated more often than the prepacked samples ($P < 0.07$). Significant differences were observed between the two time points in the survey; a higher prevalence of *Salmonella* was reported in the winter months of the survey ($P < 0.02$).

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In total, 27 *Salmonella* isolates were identified. *S. Typhimurium* accounted for 23 of the isolates. *S. Derby* was isolated on two occasions while *S. Livingstone* and *S. Bredeney* were each isolated once.

Salmonella counts on twelve samples, which were positive for *Salmonella* when first examined, ranged from 1.5 to 37 organisms per 25g of sausage meat examined. In 50% of samples the pathogen was reduced to undetectable levels (< 0.3 MPN per 25g).

Discussion: The results of this study indicate that the rate of *Salmonella* contamination in Irish retail pork sausage samples is low (<5%). The detection rate was similar to other studies, which examined pork products, where *Salmonella* contamination was detected at levels ranging from 3.3% to 9.1% (Zhao et al., 2001, Mattick et al., 2002). The difference in prevalence levels could be due in part to the types of samples analysed i.e. comminuted product versus pork chop and also whether samples were fresh or frozen.

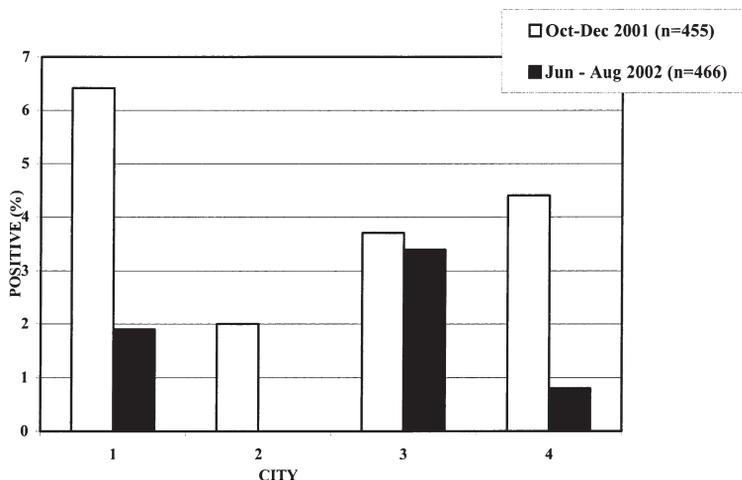
Outbreaks and clusters of human salmonellosis in Ireland show a marked seasonality with the highest number of cases occurring during the summer months (June to August). However, in this study the rate of contamination was significantly higher in winter months. No plausible explanation for this result can be offered, however similar findings were reported by Stock & Stolle, (2001) and Zhao et al., (2001).

Eighty five per cent of positive isolates recovered in this study were serotyped as Typhimurium. The predominance of *S. Typhimurium* is consistent with the results of other recent studies, which have examined pork and pork products (Stock et al., 2001). The presence of this serotype in pork is a cause of concern as *S. Typhimurium* is the top cause of human salmonellosis in Ireland.

Quantitative analysis of selected samples revealed that the number of *Salmonella* organisms in contaminated sausages was low (<1.5 to 40 per 25g). In this study pathogen levels were reduced to undetectable levels in 50% of samples. It is possible that initial counts of *Salmonella* in fresh samples were extremely low and therefore less likely to survive freezing. This survey confirms that a percentage of raw pork sausage meat in Ireland is contaminated with *Salmonella* and may lead to foodborne disease due to undercooking and/or cross contamination. The enactment into law of the National *Salmonella* control programme in Ireland (2002) should help to decrease the number of positive carcasses entering the abattoirs and thus reduce the prevalence of *Salmonella* in retail pork products.

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Figure 1. Prevalence of *Salmonella* in samples of pork sausage collected in 4 cities on two occasions



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Comparison of an excision and a sponge sampling method for measuring salmonella contamination of pig carcasses

PD 01

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Summary: The aim of this study was to determine if an excision sampling method and a sponge sampling method give comparable results when trying to isolate salmonella from pig carcasses. During ten sampling days in one abattoir in total 312 carcasses were sampled; each carcass was sampled with both sampling methods to get paired observations. The number of salmonella positive excision samples (31 of 312) was significantly higher ($P=0.00013$) than the number of salmonella positive sponge samples (9 of 312). Sensitivity of the sponge method compared to the excision method was 6.5% and the comparability of both tests was low (κ value was 0.08). As it seems that salmonella contamination levels of fresh pork are highly underestimated with the actually used sampling methods, the authors recommend that EU-authorities prescribe a destructive salmonella test for monitoring pig carcasses after slaughter in all EU-countries or a swab/sponge method with a comparable sensitivity.

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Introduction : In order to monitor the product of pig abattoirs, samples for bacteriological investigation can be taken of the end product of the abattoirs: split pig carcasses. Several methods for carcass sampling have been described (Gill and Jones, 2000, Snijders et al., 1984, Dorsa et al., 1996, Dorsa et al., 1997, Swanenburg et al., 2000). In general these methods can be divided in destructive methods, such as the cork borer method, and non-destructive methods, such as swabs, sponges and contact plates. Dutch abattoirs have to sample their carcasses regularly for the presence of salmonella to get permission to export their products to the USA. The sampling method is prescribed exactly by the American Food Safety and Inspection Service and is carried out by (or under supervision of) the State Veterinary Inspections Service for Livestock and Meat (Anonymous, 2002). On the other hand, abattoirs can use their own sampling schemes and methods for their own monitoring purposes. In this study an excision sampling method by which a piece of belly hide of the carcass was cut out was compared with the sponge sampling method, which is used for the "USA" sampling of carcasses. Aim of this study was to determine if these two sampling methods give comparable results when isolating salmonella from pig carcasses.

Materials and Methods: Samples were collected in a Dutch pig abattoir during five weeks on Tuesdays and Thursdays. On each sampling day two different samples were collected from approximately 40 randomly chosen carcasses. The excision sample was taken from carcasses, hanging from the hind legs in the line, just after veterinary inspection. The sample consisted of approximately 60 cm² of belly hide, cut from the cutting surface of the belly just above the sternum (the cutting surface of the belly originates from the opening of the belly by the belly opening saw). The contamination of this surface represents the contamination caused by machinery in the slaughterline. The sample was cut from the carcass using a sterile meat knife and a pair of tweezers and put in a sterile plastic stomacher bag. The carcass was identified to ensure that the sponge sample would be taken from the same carcass. Sponge samples were taken 24 hours after slaughter from the same carcasses as the excision samples. For the sponge sample the Meat Turkey Carcass Sampling Kit (Nasco, USA) was used. The samples were taken according to the official instructions laid down by the Dutch State Veterinary Inspections Service for Livestock and Meat (RVV) (Anonymous, 2002), which are based on the rules of the Food Safety and Inspection Service (USA, FSIS, 1996). Three areas of 100 cm² each were swabbed, one area on the belly 10 cm from the cutting surface, one area on the ham (both with one side of the sponge), and one on the jowl (with the other side of the sponge). Sponges were put in the plastic bag (Whirl-Pak) and transported to the laboratory. Salmonella was isolated from the samples according to standard procedures (Buffered Peptone Water, Tetrathionate broth, Brilliant Green Agar, confirmation with Triple Sugar Iron agar, Lysine Decarboxylase and urea-agar). Salmonella isolates were serotyped with group A-G anti-salmonella serum. Statistical analysis of data was done with Statistix 7.0.

Results: A total of 312 paired observations for both tests were made. Salmonella isolation results for both sampling methods are presented in table 1. The number of salmonella positive excision samples was significantly higher than the number of positive sponge samples (McNemar Chi square test, P=0.00013). Using the destructive sample as golden standard the sponge method had a sensitivity of 6.5% compared to the excision method. The kappa value for comparability of the two sampling methods was 0.08, where a kappa value of 0.4 is considered as a reasonable and a kappa value of 0.6 as a good comparability between tests.

Table 1. Results of salmonella isolation from sponge samples and excision samples taken from 312 pig carcasses.

| | | Result excision sample | | Total | |
|----------------------|----------|------------------------|----------|-------|--------|
| | | Positive | Negative | | |
| Result sponge sample | Positive | 2 | 4 | 6 | (1,9%) |
| | Negative | 29 | 277 | 306 | |
| Total | | 31 | 281 | 312 | (9,9%) |

Discussion: Considerably more excision samples than sponge samples were salmonella positive. It would have been logical to expect more positive results with the sponge method than with the excision method because with the sponge method a larger area was sampled and from more different locations of the carcass. On the other hand, sponge sampling was carried out 24 hours after slaughter, after the carcass had been chilled and cooled. During this time span, salmonella can attach itself to the skin or withdraw in hair follicles (Berends, 1998), and will not be removed by sponging. Furthermore, although not investigated in this study, the cutting edge of the carcass is probably more contaminated than skin surfaces as a result of contamination by contact with contaminated machinery during the slaughter process. Swanenburg et al. (2001) showed that contamination of slaughterhouse machinery during the slaughter process is largely responsible for the contamination of pig carcasses with salmonella. Although in total salmonella was isolated from more carcasses with the excision method than with the sponge method, 4 carcasses were positive when sampled with the sponge method, which were negative with the excision method. This can be explained by the fact that different locations on the carcass were sampled with both methods and at different points in time.

The results of this study show that using the internationally recognised USA sponge sampling method results in a serious underestimation of the carcass contamination in pig abattoirs. If prevalence data of carcasses, obtained with this method, are published or are used to show that the salmonella "problem" is not very large and with that suggesting that the situation is under control, this will give a false sense of security to abattoir personnel and management, customers, consumers and authorities. In our opinion more sensitive methods, as for example as described here, are more appropriate to give a clear picture of the real situation in abattoirs. Also the more detailed information gives better opportunities to focus intervention strategies more precisely and leaves more room for improvement than the very low prevalence found with (variations on) the USA-sponge-method (S. rensen et al, 2001). An advantage of the USA sponge method is that it is well described and internationally accepted in the industry. To introduce a new and much more sensitive sampling method which is comparable between studies and countries requires international agreement on the method and support from the authorities. EU-wide and preferably world wide agreement and implementation on a sensitive sampling method will make it possible to compare salmonella prevalences of carcasses between countries and pork producers.

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PD 02

Evaluation of Pooled Serum and 'Meat-Juice' in a *Salmonella* ELISA for Pig Herds

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Summary: Samples of 'meat-juice', serum, caecal contents and carcass swabs from 420 pigs from 20 finishing farms were tested for *Salmonella* bacteriologically and serologically by ELISA on individual samples or on pools of serum or meat juice. In addition pooled floor faeces were taken from the finishing pens on the farm of origin.

Salmonella was isolated in samples from 19 of the 20 farms. 32.8 % of pooled pen faeces and 24.3 % of caecal samples were positive but *Salmonella* was only found in 1.7 % of carcass swabs. 43.2 % of individual 'meat-juice' samples and 25.3 % of serum samples gave positive ELISA results. Neither the individual or pooled ELISA tests showed a statistically significant correlation with caecal carriage of *Salmonella* or contamination of carcasses, although the percentage of positive pen faecal samples did correlate significantly with caecal positives. Only serum mean optical density from pools of 5, 10 or 20 sera correlated significantly with *Salmonella* prevalence in pen faecal samples but all pooled serum and meat-juice optical density or sample/positive ratios correlated significantly with the percentage individual ELISA positives. This suggests that pooled serum or meat-juice could be used as an alternative to individual samples for ranking herds.

Keywords: serology, monitoring, comparison, swine, contamination

Introduction: Monitoring for *Salmonella* in slaughter pigs is important to enable targeted control measures to be applied on significantly infected farms and at the abattoir. Serological testing using a LPS based mix ELISA has been shown to be suitable for ranking herds according to likely weight of infection (Nielsen et al., 1998), but testing sufficient numbers of samples to obtain an accurate herd ranking is expensive for an industry in severe financial difficulties. Pooled samples are routinely used to maximise bacteriological detection of *Salmonella* with limited resources. This paper describes a study designed to assess the suitability of pooling of serum or meat juice samples for ranking the *Salmonella* status of pig herds.

Materials and Methods: Approximately 420 serum, meat juice, carcass swab, and caecal contents samples were obtained from groups of slaughter pigs from 20 farms. Carcass swabs were taken according to a US/Danish protocol. In addition, the farms were visited on the day before slaughter and pooled faeces collected from pens occupied by the pigs to be slaughtered. Serum and meat juice samples were tested by ELISA (Vetsign *Salmonella* ELISA Kit; Guildhay) as individual samples and as pools comprising 5, 10 and 20 individual subsamples. Bacteriological culture carried out by 18 hours pre-enrichment at 37 °C Buffered Peptone Water (BPW; Merck), 48 hours selective enrichment at 41.5 °C in DIASALM medium (Merck), with subculturing on to Rambach agar (Merck) plates after 24 and 48 hours culture. The plates

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were incubated for 24 hours at 37 °C and suspect colonies confirmed by standard biochemical and serological tests. Serotyping and phage typing was carried out by VLA Weybridge. Statistical analyses were made on the basis of herd results and correlations and descriptive statistics calculated with Statistica (StatSoft, Inc. 2001).

Results: 109 (25.3 %) of the 430 serum samples taken were positive (sample/positive ratio (SP) > 0.25), placing 7 herds in the equivalent of Danish Category 1, 11 herds in Category 2, and 2 herds in Category 3, ie. the worst *Salmonella* status category, requiring further action. The mean SPs from the various poolings were 0.33 (0.08-0.83), 0.33 (0.05-1.19) and 0.37 (0.07-1.39) for pools of 5, 10 and 20 respectively. 182 (43.2 %) of 421 meat-juice samples were positive, which would place 8 of the 20 farms in Category 3. The mean SP results from the various poolings were slightly higher than the mean of the individual samples; 0.41 for individuals, 0.48 for pools of 5, 0.51 for pools of 10 and 0.48 for pools of 20.

7 (1.7 %, herd range 0-9.1 %) of 422 carcass swabs contained *Salmonella*. The positive carcass swabs originated largely, but not exclusively, from herds with high numbers of caecal positives. 102 (24.3 %) of 420 caecal samples contained *Salmonella*, predominately S.Typhimurium (17.4 % samples) which were mostly DT104 or related strains such as U302. 121 (32.8 %) of 369 pooled pen faeces samples contained *Salmonella*. The serotypes and phage types corresponded well with those found in caecal contents. No *Salmonella* or ELISA positives were found in one of the 20 farms.

None of the ELISA tests, either % positive individual ELISA tests or optimal density (OD) or SPs for pooled sera correlated with caecal *Salmonella*. Optimal density for pools of 5, 10 and 20 sera correlated significantly with the % positive farm pens, but there was no such correlation for 'meat-juice'. For all pools the ODs and SPs correlated significantly with % positive individual samples and there was a significant correlation between the % positive farm pen samples and % positive caeca. Although there was a significant correlation between serum and meat-juice results there were substantially high numbers of positive samples with meat-juice.

Discussion: This study demonstrates that pooled serum or meat-juice could be used for ranking pig herds as part of a *Salmonella* surveillance and control programme. More work is required to refine the application of the test, and in particular more long-term studies to establish herd serological and bacteriological profiles and appropriate weighting factors for recent versus earlier herd results. The application of the test would probably be more effective if a percentage of 'worst' herds for further action was applied rather than using fixed banding categories. It is clear also that the correlation between test results from serum and meat-juice could be improved by attention to dilution factors.

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PD 03 Real-time PCR assays for the detection of *Mycoplasma hyopneumoniae* in clinical samples

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Summary: Two real-time PCR assays for detection of *Mycoplasma hyopneumoniae* (*Mhyop*) in clinical lung samples were established and validated in parallel. One is targeting a repetitive DNA element (REP assay) the other a putative ABC transporter gene (ABC assay). The two assays were shown to be 100% specific when testing pig lungs from defined negative farms. When investigating defined positive farms the REP assay tested with a sensitivity of about 50%, the ABC assay with 90%. The two assays together, however detected 100% of positive farms. Within a single positive farm on average 90% of the samples tested positive with the REP or ABC assay. Analysing a set of 41 lungs from infected pigs from routine diagnostic the REP assay detected 50% and the ABC assay 70%, while both assays together had a sensitivity of 85%.

Keywords: enzootic pneumonia, pig disease, diagnostic, TaqMan, eradication

Introduction: The current diagnosis of enzootic pneumonia (EP) of swine is based on a polyphasic approach integrating various aspects of the disease. These include clinical signs, serology, pathological investigation of lungs and detection of the pathogen *Mhyop*. The latter can be done by immunofluorescence, by Giemsa staining or by culture. The PCR technology and particularly the real-time PCR (rtPCR) would be the method of choice for pathogen detection, since some unspecific cross-reaction with other mycoplasmas was observed with immunofluorescence, Giemsa staining is of low specificity and culture is fastidious and time consuming. Several PCR assays have been developed on specific *Mhyop* DNA fragments, including two nested-PCR (Stärk et al., 1998; Verdin et al., 2000). Nevertheless, this kind of assay is not convenient for routine diagnosis of EP. Therefore, we developed and validated two rtPCR methods for the routine detection of *Mhyop* in clinical lung samples.

Materials and Methods: A total of 208 lungs obtained from slaughterhouses were analysed in this study. After macroscopic assessment and sampling for histological analysis lungs were forwarded for further bacteriological examination. In order to have defined positive and negative sample groups and to analyse several samples from a defined case, we selected 16 farms with clearly defined sanitary status regarding EP (11 EP-positive and 5 EP-negative farms with normally 10 lungs/farm). The farm status definition was based on the presence or absence of clinical signs (mainly chronic, non-productive coughing), epidemiological tracing of the farm, typical necropsy and histopathological lesions and positive immunofluorescence results at the farm level, which together represent a so called "mosaic diagnosis". In addition, 70 lungs were from routine EP diagnosis and were selected by pathologists according to clinical and macroscopic signs. These single lungs were collected out of several typical lungs of animals from the same farm. Bronchial swabs were taken and a lysate was prepared for PCR detection of *Mhyop*. DNA sequencing of part of the two target genes was done from several *Mhyop* probes. The REP assay targeting a repetitive DNA element is using a classical TaqMan[®] probe to detect and specify the amplicon. The ABC assay targeting a putative ABC transporter gene uses a shorter TaqMan[®] MGB probe. The rtPCR were established using several bacterial and mollicutes DNA before they were validated on clinical lung material.

Results: Sequence variations in both target genes were observed and considered when choosing primers and probes. Both rtPCR assays tested positive with *Mhyop* strains used and were always

negative with DNA from other mollicutes and bacteria commonly isolated from pig. Within the EP-negative set consisting of 44 samples from 5 farms both rtPCR tested negative for all samples (100% specificity). For the EP-positive set 94 samples obtained from 11 farms were tested. The REP assay tested positive on 44 samples out of 6 farms (sensitivity of 48.35 +/-10.27%, p<0.05). Within these REP positive farms the assay tested positive between 80-100% (mean 93.33%) of the lungs originating from the same herd. Five farms were negative in the REP assay for all individual lung samples tested. The ABC assay tested positive on 77 samples from 10 farms (sensitivity of 82.80 +/-7.67%, p<0.05). Within these ABC positive farms the assay tested positive between 60-100% (mean 95.00%). The assay tested negative for all 10 samples collected from 1 farm. The two rtPCR assays together showed positive for all the 11 positive farms and the results within a positive farm were clearly positive (on average >90% of the individual samples). Five farms were negative with the REP assay but positive with the ABC assay. On the other side the single farm testing negative with the ABC assay gave 100% positive results with the REP assay. For further evaluation of the two rtPCR 70 single routine diagnostic cases were collected and assayed by rtPCR. These samples were retrospectively defined as consisting of 41 EP-positive and 29 EP-negative samples by the classical "mosaic diagnosis". For the 29 negative samples both the REP and ABC assays tested negative (100% specificity). Out of the 41 EP-positive diagnostic samples 21 were positive in the REP assay (sensitivity of 51.22 +/-15.30%, p<0.05). 12 REP-negative samples were positive in the ABC assay. The ABC assay tested positive on 28 samples (sensitivity of 68.29 +/-14.24%, p<0.05). 5 ABC-negative samples were positive in REP. The calculated sensitivity obtained by the use of both rtPCR assays in parallel was 84.52%.

Discussion: The rtPCR assays developed and validated are a valuable tool for diagnosis of EP, what is especially important in the light of a solid support for decisions being taken for the eradication of the disease. The assay is very specific and sensitive on the herd level. Analyzing single probes from suspicious herds as done in routine diagnosis might be prone to false negative results, as the findings with the diagnostic samples indicate. We recommend to take at least lungs from 3 individuals in such cases. PCR detection as a method is less dependent on subjective interpretation compared to the immunofluorescence or pathological examinations. This together with the speed and large sample numbers that can be tested by rtPCR makes it the method of choice, moreover since it is less sensible to contamination. In order to increase the overall sensitivity of the method it would be useful to develop a multiplex assay, which allows to carry out both the REP and ABC assay in a single tube.

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PD 04

Use of recombinant ApxIV in serodiagnosis of *Actinobacillus pleuropneumoniae* infections and development of an ApxIV ELISA

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Summary: *Actinobacillus pleuropneumoniae* (*App*) is the etiological agent of porcine pleuropneumonia. The virulence of the fifteen serotypes of *App* is mainly determined by the three major RTX toxins ApxI, ApxII and ApxIII, which are secreted by the different serotypes in various combinations. A fourth RTX toxin, ApxIV, is produced by all 15 serotypes specifically during infection of pigs, but not under in vitro conditions. Pigs infected with *App* show specific antibodies directed against ApxIV. The analysis of sera of experimentally infected pigs revealed that ApxIV-immunoblots detected *App* infections in the second to third week post infection. We developed an indirect ELISA based on purified recombinant N'-terminal moiety of ApxIV that showed a specificity of 100% and a sensitivity of 93.8%. The pre-validation study of the ApxIV-ELISA revealed that the latter was able to detect *App*-positive herds, even if clinical and pathological signs of porcine pleuropneumonia were not evident.

Keywords: serodiagnosis, ELISA, immunoblot, Apx toxin, seroconversion

Introduction: Surveillance of *App* infections can be done by serological examination of pig herds. For a few selected serotypes such as serotype 2 and the group of serotypes 1-9-11, ELISA kits are currently available on the market. Recently other serotypes such as serotype 7 and/or 12 are emerging in Switzerland and were also detected in other parts of Europe. For such serotypes, there are no commercial ELISA tests available. Apx toxins are highly immunogenic and thus induce a strong production of antibodies in animals infected with *App*. However, ApxI, ApxII and ApxIII are also secreted by other *Actinobacillus* species like *A. rossii*, *A. suis*, and the recently discovered, non-pathogenic "*Actinobacillus porcitonissillarum*" which can induce antibodies against these toxins in pigs, whereas ApxIV is specific to the species of *App* (Schaller et al., 2001). The present study aims to assess the seroconversion against ApxIV in swine with porcine pleuropneumonia using immunoblot analysis and to develop and pre-validate an ApxIV-based indirect ELISA for *App*.

Materials and Methods: Recombinant NiApxIV was used on immunoblots (IB) to serologically detect antibodies against ApxIV in *App* infected pigs. Subsequently an indirect ELISA based on NiApxIV was developed. Cut off level, sensitivity and specificity were determined by testing positive sera of experimentally infected pigs, pig herds naturally infected by *App* and negative sera of specific pathogen free (SPF) and *App*-free herds. These sera were tested by NiApxIV-IB and by LPS ELISA. Furthermore, the lungs and tonsils of the animals, from which the sera originated were tested by PCR and/or by isolation of *App*. *App*-positive and negative field sera were tested by NiApxIV-IB and ELISA. Seroconversion against ApxI, ApxII and ApxIII was examined, by testing sera of *App*-positive and negative herds by IB.

Results: A significantly higher number of animals showed reactions towards ApxI, ApxII and ApxIII than towards ApxIV. In particular sera from herds that were free of porcine pleuropneumoniae showed immunological reactions to one or several of ApxI, ApxII or ApxIII but not ApxIV. Pigs with known history of *App* infections constantly showed anti ApxIV antibodies. The progress of the immune response to ApxIV investigated by analysis of sera from pigs experimentally infected with *App* serotype 9 showed seroconversion to ApxIV between 20-28 days p.i., as indicated by immunoblot analysis and measured by ApxIV-ELISA with continued increase. In order to pre-validate an ApxIV-ELISA, field sera of pigs

originating from different herds with problems of porcine pleuropneumonia and/or confirmed *App* infections, as determined by isolation of *App* or serological diagnosis using currently available tests, were analysed by ApxIV-ELISA. In addition, sera from slaughterhouse pigs with typical lung lesions were scored by the ApxIV-ELISA. Thereof, 45 sera (52 %) were positive. Among the 86 slaughterhouse sera, 35 samples originated from pigs of which *App* was isolated from lungs. Among these 35 sera, 16 samples (46%) tested were positive in the ApxIV-ELISA, 15 (43%) were negative, and 4 (11%) showed ELISA titers close to the cut-off value. Out of the remaining 51 slaughterhouse sera of pigs, from which *App* was not isolated by standard procedures, 29 (57%) gave positive results, 20 (39%) gave negative results, and 2 (4%) were close to the cut-off value. In the negative sampling of sera used for the pre-validation, totally 52 sera from 40 pigs and 12 sows from herds known to be free of *App* infections showed to be negative in the ApxIV-ELISA.

Discussion: This study shows that immunoblot and indirect ELISA detect antibodies against ApxIV in *App* infected pigs 20 days post infection. It reveals that the ApxIV ELISA is a highly specific and sensitive test to detect seroconversion against ApxIV in pig herds infected by *App*. The specificity of the ApxIV-ELISA was shown to be very high since no sera from pigs and sows from *App*-free herds gave ELISA titers above the cut-off value. Furthermore it must be noted that sera from pigs experimentally infected with the non-pathogenic *Actinobacillus* species "A. porcitonisillarum", were negative in the ApxIV-ELISA although this new bacterium caused sero-conversion to LPS capsular antigens of *App*, a fact that severely hampered serotype-specific ELISA under certain conditions (Gottschalk et al., 2003) . It further supports other studies in the observation that ApxI, ApxII and ApxIII are not specific enough for sero diagnostic use. The relatively high number of serological reactions to ApxII and ApxIII in pigs that were free of *App* is presumably due to other *Actinobacillus* species such as *A. suis* and *A. rossii*, and the recently discovered "Actinobacillus porcitonisillarum" (Schaller et al., 2000; Frey and Kuhnert 2002; Gottschalk et al., 2003). We conclude from the present study that the ApxIV-ELISA is a highly sensitive and specific serological test for the diagnosis of infections of pigs and pig herds with any of the serotypes of *App*. Thus, this test is promising for reliable surveillance and control of *App* infection.

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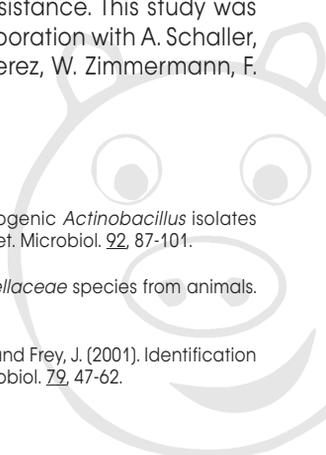
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PD 05

Stool processing-methods for *Salmonella enterica* isolation and PCR detection

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Summary: The aim of this study was to compare the efficiency of three protocols for bacteriological isolation of *Salmonella enterica* and detection by PCR in swine feces samples. Pool of feces (n=62) were processed by three different methods. Method 1: samples (10g) were pre-enriched in BPW (1:10) and enriched in Rappaport-Vassiliadis broth (1:100). Method 2: samples (1g) were first enriched in GN-Hajna broth (1:10) and secondly enriched in Muller-Kaufmann tetrathionate broth (1:10). Method 3: Single step enrichment of feces (1g) in selenite-cystine broth (1:10). PCR was performed using DNA extracted from the last enrichment broth of each bacteriological method. *Salmonella enterica* was cultured from 13 out of 62 samples (20.9%) and seven different serotypes were isolated. The methods 1, 2 and 3 resulted in 9 (14.5%), 6 (9.6%) and 2 (3.2%) positive samples, respectively. PCR was significantly superior than conventional bacteriology for *Salmonella* detection only when Rappaport-Vassiliadis was used for DNA-template preparation.

Keywords: Detection, enrichment, pigs, Rappaport-Vassiliadis, swine

Introduction: Conventional bacteriology still is the basis of epidemiological studies of *Salmonella*. However, *Salmonella* serovars are not detectable in certain clinical samples that contain small numbers of organisms. PCR is a powerful molecular biology tool for the detection of target DNA, but its application to fecal samples has been very limited because of the presence of unknown PCR inhibitors (Lou et al., 1997). The use of PCR coupled to selective enriched broths has been considered a feasible alternative to improve the sensitivity of the PCR from clinical samples because they dilute inhibitors and allow the increase of *Salmonella* organisms in the sample. The aim of this study was to compare three bacteriological protocols for *Salmonella* isolation. Additionally, the effectiveness of PCR coupled to the last enrichment broth of each protocol was also evaluated.

Material and Methods: Pool of feces (n=62) were collected from holding pens in a slaughter-house. Samples were collected using sterile plastic bags and taken to the lab under refrigeration to be processed in the same day. After homogenization, each sample was submitted to three isolation methods. Method 1 (M1): feces samples (10g) were pre-enriched in BPW 2% (1:10), incubated overnight at 37AC and transferred (1:100) to Rappaport-Vassiliadis broth (RV), which was incubated at 42AC for 24 hours. Method 2 (M2): feces samples (1g) were first enriched in GN-Hajna broth (1:10), incubated for 24 hours at 37AC and transferred (1:10) to Muller-Kaufmann tetrathionate broth (TT), which was incubated at 37AC for 48 hours. Method 3 (M3): single step enrichment of feces (1g) into 10 mL selenite-cystine broth (SC) for 24 hours at 37AC. After enrichment, a loopful of each tube was streaked onto XLT agar plates. After biochemical analysis, colonies were confirmed as *Salmonella* by slide agglutination test. Aliquots (1mL) of each enriched broth were taken and submitted to DNA extraction by a boiling-centrifugation technique. (Soumet et al., 1994). PCR was performed using specific primers for the *Salmonella* genus (S18-S19). Results were analyzed by McNemar's test for matched samples.

Results: *Salmonella enterica* was cultured from 13 out of 62 samples (20.9%) and seven serotypes were isolated: S. London, S. Lexington, S. Schwarzengrund, S. Mbandaka, S. Hadar, S. 1,3,19:-: and S.

Senftenberg. M1 resulted in 9 (14.5%) positive samples, which was superior ($P < 0.05$) than M2 (6, 9.6%) and M3 (2, 3.2%), respectively.

PCR was superior ($P < 0.01$) than conventional bacteriology only when RV was used as the DNA source. PCR using RV detected 27 out of 62 samples (43.5%), which was superior ($P < 0.01$) than using SC or TT (3.2% and 8.0%, respectively).

Discussion: Considering the effect of sample weight on the detection of *Salmonella* bacteria (Davies et al., 2000), we cannot directly compare the three enrichment media used herein. However, we suggest the use of M1 whenever possible to obtain large amounts of fecal material (10g). Furthermore, the use of direct enrichment of swine feces in SC is discouraged.

The coupling of RV to PCR was significantly superior ($P < 0.01$) than either TT or SC. Indeed, RV has been successfully used for PCR purposes in poultry-related samples (Oliveira et al., 2002). The best results of M1 (which used RV as enrichment broth) for isolation of *Salmonella* could indicate that this broth yielded higher numbers of *Salmonella* organisms, which could also explain the superior PCR results for RV. However, selective enrichment media may have different inhibitory properties to PCR. In fact, tetrathionate broth has been suggested to inhibit PCR (Stone et al., 1994).

Our results indicate that RV broth may be successfully coupled to PCR after a simple boiling-centrifugation technique for DNA extraction. This is of great importance considering the cost-benefit ratio of this procedure.

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COMPARISON OF ENRICHMENT SCHEMES FOR THE ISOLATION OF *YERSINIA ENTEROCOLITICA*

PD 06

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Summary: The food-borne pathogen *Yersinia enterocolitica* (YE) has been repeatedly linked to swine and is a world wide food safety risk. Microbiological culture methods for YE lack some functionality as the current gold standard requires a 21 day cold enrichment in phosphate buffered saline (PBS). In this study a shortened enrichment scheme using a higher incubation temperature and a more selective media (LB-BSI) was compared to PBS for the isolation of YE from swine feces. Both enrichments

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produced similar overall isolation rates but had a low level of agreement ($\kappa=0.11$) for determining an individual pig's YE status. LB-BSI yielded a sensitivity and specificity of 22.7% and 87.2% respectively when compared to PBS. At the time of submission, further genotyping and serotyping assays are pending that may aid in discerning the source of disagreement between the methods.

Keywords: Sensitivity, specificity, feces, PBS, LB-BSI

Introduction: *Yersinia enterocolitica* (YE) is a food-borne pathogen causing an estimated 96,000 Americans to become ill each year (Mead, 1999). While the bacterium has been found in a variety of food and environmental samples, swine and pork products have been implicated as the primary reservoir of pathogenic YE (Davies, 1997). Fecal culture provides a relatively easy means to screen live pigs for the presence of YE. However, the standard methodology requires a three week wait for a negative result. Several enrichment schemes have been developed to shorten the wait time and aid in the isolation of YE from various sources (Fredriksson-Ahomaa, 2003). The aim of the study was to evaluate the effectiveness of Luria-Bertani-Bile Salts-Irgasan broth (LB-BSI) as a selective enrichment method for the isolation of YE from naturally infected swine feces as compared to the gold standard of cold enrichment in phosphate buffered saline (PBS). LB-BSI is advantageous compared to cold enrichment in PBS due to markedly shorter incubation time. Previous studies have not compared the methods in naturally infected feces.

Materials and Methods: During February to June 2003, 379 individual pig fecal samples representing 10 farms in Ohio and North Carolina were collected. Pigs included in this study ranged in age from 9 weeks to mature sows. Twenty gram fecal samples were collected by gloved hand from the rectum of each animal, placed in sterile Whirl-paks™ (Nasco, Fort Atkinson, WI, USA), and transported to the laboratory on ice. Samples were stored at 4°C for up to 24 hours until they were processed. Each 20 g sample was split into two 10g sub samples and placed in sterile specimen cups for enrichment in treatment A or B. Treatment A was the gold standard three week cold enrichment in PBS (Aleksic, 1999). Briefly, the 10 g sub samples were homogenized with 90 ml of 1X PBS (EMD Chemicals Inc., Gibbstown, NJ, USA) and incubated for 3 weeks at 4 °C before being plated. Treatment B was the Luria Bertani-Bile Salts-Irgasan (LB-BSI) selective enrichment based the methods of Bhaduri et al. (1997) and Hussein et al.(2001). Treatment B samples were diluted at a ratio of 1:10, homogenized, and incubated at 12 °C for 24 hours. After 24 hours, Irgasan (Ciba-Geigy Corp., Greensboro, NC, USA) was added at a final concentration of 5 µg/mL and re-incubated at 12 °C for an additional 48 hours before being plated. Following enrichment, 10 µL of each broth was streaked on separate Yersinia Selective Agar, CIN plates (Becton Dickinson and Company). Plates were incubated at room temperature for 48 hours. Colonies with morphology typical of YE were further biochemically identified by subculture onto Kligler Iron Agar (KIA, Becton Dickinson and Company) and urease broth (Becton Dickinson and Company). Colonies that fermented dextrose only and were urease positive were classified as presumptive YE. Presumptive isolates were stored at -80 °C for further characterization.

Results: At the time of the submission, all 379 samples have been processed through both treatments to the point of biochemical confirmation. The number of pigs found to be positive by the PBS and LB-BSI methods was 17.4% and 14.5% respectively. While the two methods detected a similar prevalence of YE there was lack of agreement between the two methods concerning YE status of individual pigs ($\kappa = 0.11$, Table 1). Sensitivity was 22.7% and the specificity was 87.2% when comparing LB-BSI to the PBS cold enrichment.

Discussion: Given that the two enrichment schemes yielded similar prevalence rates for isolation of YE, yet had poor agreement at the pig level, the source of the disagreement needs further investigation. The stored isolates will be assayed for the presence of *ail*, a chromosomally encoded virulence gene associated with human clinical disease isolates, in order to assess the possibility of differential selection between the 2 methods for *ail*-harboring and non-*ail* harboring isolates. Additionally, the YE isolates

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will be serotyped to assess agreement between the methods for isolation of different serotypes. Serotyping and PCR results of the isolates may elucidate whether the disagreement between LB-BSI and PBS is a function of lesser sensitivity or greater inhibition of non-pathogenic YE.

Table 1: Isolation of *Y. enterocolitica* from swine feces by PBS and LB-BSI

| | | PBS | | TOTAL |
|--------|-----|-----|-----|-------|
| | | Yes | No | |
| LB-BSI | Yes | 15 | 40 | 55 |
| | No | 51 | 273 | 324 |
| TOTAL | | 66 | 313 | 379 |

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CHARACTERISATION OF *SALMONELLA CHOLERAESUIS* BY PFGE AND RIBOTYPING

PD 07

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Summary: *Salmonella* (S.) Choleraesuis var. Kunzendorf is the major cause of swine salmonellosis in Poland. Eleven *Xba*I macrorestriction profiles and 3 ribotypes were noted amongst 36 isolates tested. Index of discrimination reached 0.88. Two clonal lineages were distinguished. One of the lines embraced the majority of 2000-2002 isolates showing over 80% genome similarity. The findings prove the clonal spread of the pathogen among swineherds in Poland.

Keywords: swine, salmonellosis, molecular typing, FIGE

Materials and methods: Thirty-six *S. Choleraesuis* var. Kunzendorf strains isolated from swine salmonellosis during 2000-2002 were genotyped by means of PFGE - Field Inversion Gel Electrophoresis (Hoeffer Scientific Instruments) and ribotyping. DNA samples were prepared as suggested by PulseNet

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(Swaminathan B. et al., 2001). *Xba*I macrorestriction digest was run at 6 V/cm, 10°C, F:R 3:1 in three-cycle program (pulse I: 3,0-99,0 s – 8 h; pulse II: 0,3-30,0 s – 12 h; pulse III: 0,1-10,0 s – 8 h). "Salmonella Braenderup H9812 Global Standards" *Xba*I digest was used as a molecular weight marker. Gel-Doc 2000 (Bio-rad) was used for image acquisition and Bio-Profil software (Vilber Lourmat) for similarity analysis (UPGMA, Dice, 2% tolerance). Southern blot hybridisation was used for ribotyping. *Eco*RI digest was run in 1% agarose (2V/cm, 17h). The gels were blotted to nylon membrane by capillary procedure. DIG-labelled probe (random primed *E. coli* 16S and 23S rRNA) was prepared and used for hybridisation according to "The DIG System User's Guide for Filter Hybridization (Boehringer). DIG-labelled *I*/HindIII (Boehringer) was applied as a molecular weight marker. The ribotypes were visualised with chemiluminescence method. Index of discrimination was calculated according to (Hunter, P. R. and Gaston, M. A., 1988). Contingency test at 0.5 significance was used for statistical purposes.

Results: Of eleven macrorestriction profiles noted the most frequent were Ch/X01 (n = 10), Ch/X02 (n = 9) and Ch/X03 (n = 7). The remaining profiles were found in two or single isolates (figure 1). Ten of the profiles were related in at least 84%. The isolates of this cluster belonged to two ribotypes (R-1 and R-2) that differed by single band size. Ribotype R-2 gathered 4 strains (1 isolate of Ch/X01 and 3 isolates of Ch/X02 profile). Ribotype R-1 was observed in the remaining 31 isolates. The mentioned profiles predominated among 2000/2001 isolates and Ch/X03 was most frequently found in 2002 (p Θ 0.05). A single isolate developed both unique (Ch/X23) macrorestriction profile showing 67% of similarity to the remaining strains, as well as the ribotype with major differences to the previous ones (6 band difference). The discriminatory power of both methods used reached 0.88.

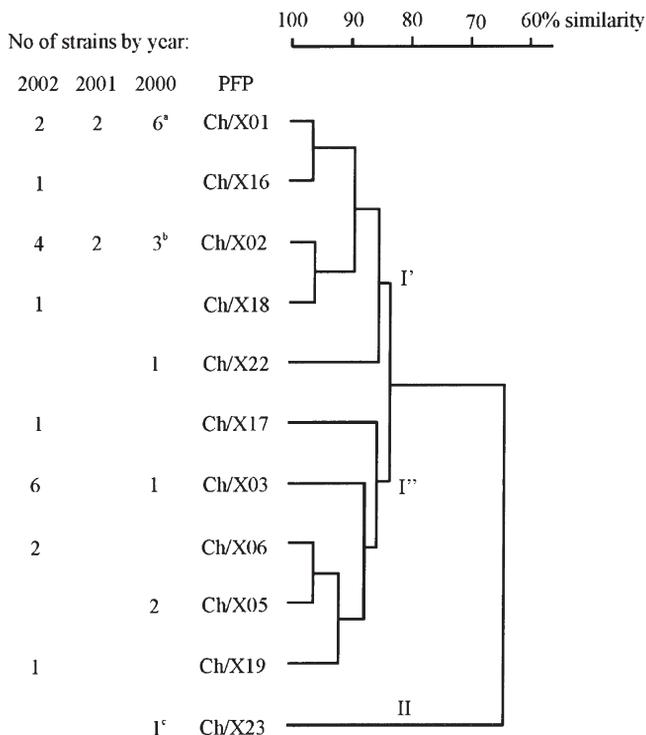


Figure 1. Phylogenetic relationship of *S. Choleraesuis* – distribution of macrorestriction profile by year and ribotype (° – one of the six strains showing R-2 ribotype; ^a – three strains of ribotype R-2; ^b – single isolate of ribotype R-3; the remaining strains belonged to the predominant ribotype R-1)

Discussion: A host specific *Salmonella* Choleraesuis remains the prevalent serovar in Poland and some other EU Candidate Countries as well as in the US (Hoszowski A. and Wasyl D., 2002; Weide-Botjes, M. et al., 1996). *S. Choleraesuis* var. Kunzendorf is responsible for the majority of the clinical salmonellosis in swine including acute septicaemia and pneumonia in piglets and fattening pigs. It causes up to 80% of *Salmonella* infections and more than 90% cases of clinical disease in Poland. The aim of present study was to determine the genetic relatedness of recent *S. Choleraesuis* isolates. The tested strains belonged to two clonal lineages. One of them (II) was represented by single strain isolated in 2000 and it was not found in pigs any longer. The other line spread over three years within animal population. High similarity of macrorestriction profiles and ribotypes indicate the affinity of the strains (Weide-Botjes, M., Liebisch, B., Schwarz, S., and Watts, J. L., 1996) and suggest clonal spread of the pathogen among swine in Poland. Some shift of predominant macrorestriction profiles and ribotypes was noted. Sub-cluster (I') gathering mostly strains of Ch/X01 and Ch/X02 profiles predominated during first part of the three-year period. The second sub-cluster (I'') covered isolates showing mostly Ch/X03 profile was more often found in 2002. All isolates presenting R-2 and R-3ribotypes were obtained in 2000. Either the macrorestriction profiles and the ribotypes revealed limited number of band differences that suggest that only a few genetic events took place during the spread of the clone (Tenover F.C. et al., 1995). The findings emphasise conservative character of *S. Choleraesuis*. Further long-time studies are needed to verify that thesis as well as to point out the possible crucial role of symptomless carriers or subclinical infected adult pigs for the disease spread among herds.

Conclusions: A low genetic divergence of *S. Choleraesuis* strains is concluded. It proves the clonal spread of *S. Choleraesuis* in Poland. PFGE and ribotyping proved to be useful and discriminative methods for *S. Choleraesuis* differentiation.

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Comparison of two commercial ELISA for the diagnosis of salmonellosis in swine

PD 08

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Summary: Three hundred and sixty-one pig sera collected in farms of Catalonia were randomly selected from a serum bank. Samples were examined by using two commercial ELISA kits. Results were compared with the κ value using WinEpiscope. Besides, sample/positive ratios (S/P) were

calculated. Comparison of results of both ELISA yielded a poor agreement (κ 0.191), indicating that both ELISA did not measure the same. In addition, when raw optical densities were compared by means of a regression analysis, the results indicated a low correlation ($r = 0.54$). The results of this study clearly indicate that results of both kits are not interchangeable and that normalization of results by using S/P ratios did not serve to improve the agreement between tests. From our results, it is tempting to suggest that Salmotype detects a greater number of IgM positive pigs. The nature of these IgMs (salmonella-specific or not) is not known to us at this moment

Keywords: Salmonella, κ , Serology, Agreement, Farms

Introduction: Salmonellosis is one of the leading causes of human infectious enteritis worldwide. Poultry meat and eggs are thought to be the main source of human salmonellosis although several studies pointed out that pigs can have a considerable role in this sense. As a result, in several countries control programmes of pig salmonellosis are being implemented. Most of these programmes use ELISA as serologic diagnostic tool because of its reliability and simplicity of use. However, some discrepancies have been noted between different ELISA systems (Van de Heijden, *et al.*, 2001). The purpose of the present study was to compare two ELISA kits routinely used in Europe for the diagnosis of swine salmonellosis.

Material and methods: Three hundred and eighty two pig sera collected in farms of Catalonia were randomly selected from a serum bank. This sample represented 194 fattening pigs and 167 sows. All animals come from farms of known Salmonella status (positive or negative) as determined by previous serological or bacteriological analysis. Samples were examined by using two commercial ELISA kits Salmonella-abB (Svanova Biotech, Uppsala, Sweden) and SalmotypeB (Labor Diagnostik, Leipzig, Germany). The ELISAs were performed according to the manufacturer directions and results were interpreted accordingly. Categorized results (positive versus negative) were compared with the κ value using WinEpiscope software. Besides, raw optical densities were used to calculate sample/positive ratios (S/P) following this formula: $S/P = (DO_{\text{sample}} - DO_{\text{-ve control}}) / (DO_{\text{+ve control}} - DO_{\text{-ve control}})$. These S/P ratios were used in a ROC analysis to determine the S/P value that better fitted the manufacturer's criteria for classification of results. All statistical analysis were done using StatsDirect

Results and Discussion: Comparison of results of both ELISA yielded a poor agreement. Thus κ value obtained for comparisons of results using manufacturer's directions was 0.191 (confidence intervals at 95 %: 0.089 - 0.294), indicating that both ELISA did not measure the same (Table 1). In addition, when raw optical densities were compared by means of a regression analysis, the results indicated a low correlation ($r = 0.546814$, 95 % confidence interval (Fisher's z transformed) = 0.470131 to 0.615304)

Table 1. Comparison of results between Salmonella-ab and Salmotype ELISA kits

| | Salmotype + | Salmotype - |
|-----------------|-------------|-------------|
| Salmonella-ab + | 118 | 62 |
| Salmonella-ab - | 84 | 97 |

Besides, sample/positive ratios (S/P) were calculated and comparison of results between both ELISAs using S/P ratios did not improve significantly the κ -value. To determine possible causes for this discrepancy, 10 randomly selected positive sera in Salmotype were heat inactivated (56 °C, 30 min) and re-analysed with this ELISA. All sera became negative after this treatment. In contrast, positive sera in Salmonella-ab were not affected by this treatment. When S/P ratios were used, ROC analysis showed that, in Salmonella-ab, a S/P cut-off $\geq 0,3389$ agreed 100 % with the negative results obtained

using the directions and 99.44 % with the positive results. Similarly, in Salmotype, a S/P cut-off ≥ 1.3776 agreed 99.37 % with the negatives using kit directions and 99.45 % with positives.

The results of this study clearly indicate that results of both kits are not interchangeable and that normalisation of results by using S/P ratios did not serve to improve the agreement between tests. From our results, it is tempting to suggest that Salmotype detects a greater number of IgM positive pigs. The nature of these IgMs (salmonella-specific or not) is not known to us at this moment.

Reference:

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SALMONELLA SEROLOGY – WHICH SAMPLES SHOULD BE USED: COMPARISON OF MEATJUICE AND SERUM SAMPLES OF THE SAME PIGS

PD 09

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Contamination of pork with *Salmonella typhimurium* is a potential source for fatal food born Salmonella-infections in humans.

Screening programs are used in a number of countries to categorize pig farms into 3-4 Salmonella-risk-categories. A similar program will soon be implemented by the German government as well. A number of commercial ELISA-Testkits are registered in Germany for serum as well as meat juice samples. However little information is available regarding the question whether meat juice or serum samples of the same pigs will lead to the same results has not been investigated thoroughly.

Purpose

The investigation was performed to clarify if serum and meat juice samples from the same animal and taken at the same day would deliver comparable ELISA-results. Furthermore with a series of consecutive blood samplings on the same animals the time-effect on ELISA-results was to be investigated.

Methods

Random samples originated from two different slaughterhouses. Blood was taken immediately after the killing process and transported. Meat samples (1x1x1 cm) were taken from the ?????? after evisceration of the carcasses. The meat was frozen and thawed in meatjuice-sampling tubes (Firma). A commercial ELISA test (Enterisol® Salmonellen-Diagnostikum, Boehringer Ingelheim Vetmedica GmbH), a mixed-ELISA based on the polysaccharide fraction of Salmonella-O-Antigen (1, 4, 5, 6, 7, 12) was used according to the test instructions. For the longitudinal study the same, randomly selected finisher pigs of one farm were sampled 3 times at different time points (jugular vein). All samples were tested with the same Testkitbatch at the same day.

Results

Slaughterhouse 1 (Graph 1)

PP-values of samples originating from slaughterhouse 1 showed a very good correlation between serum and meat juice (Graph 1). Not only were the qualitative ELISA-results all the same between the matching samples but also the quantitative results (PP-values) were almost identical in most of the cases.

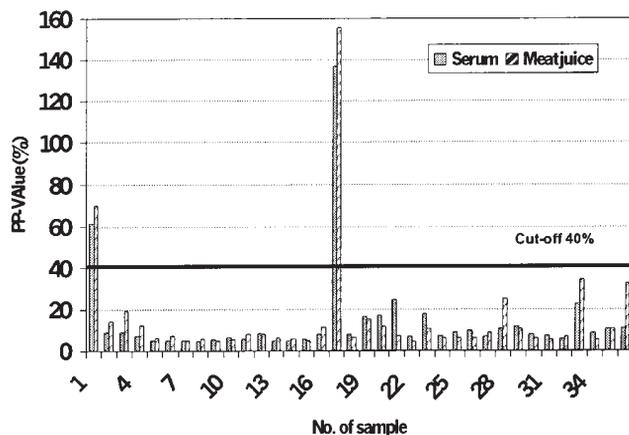
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Slaughterhouse 2 (Graph 2)

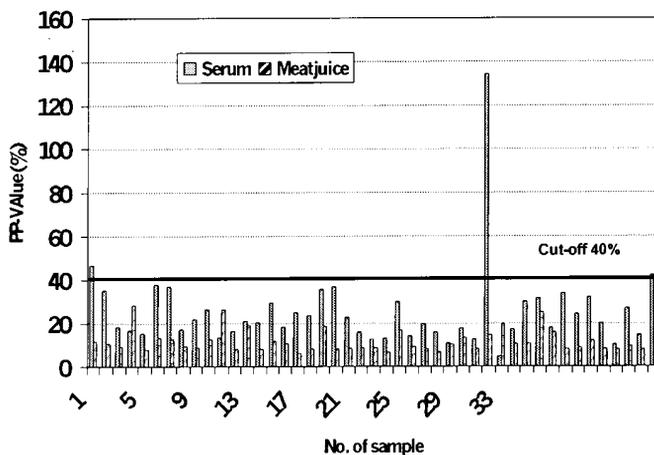
Again a fairly good correlation can be seen between the PP-values of the matching samples (Graph 2).

However some sample-pairs differed considerably and generally the serum-samples showed a higher PP-value compared to the meat juice samples.

Graph 1: ELISA results of blood and serum samples taken from the same pigs at slaughterhouse 1



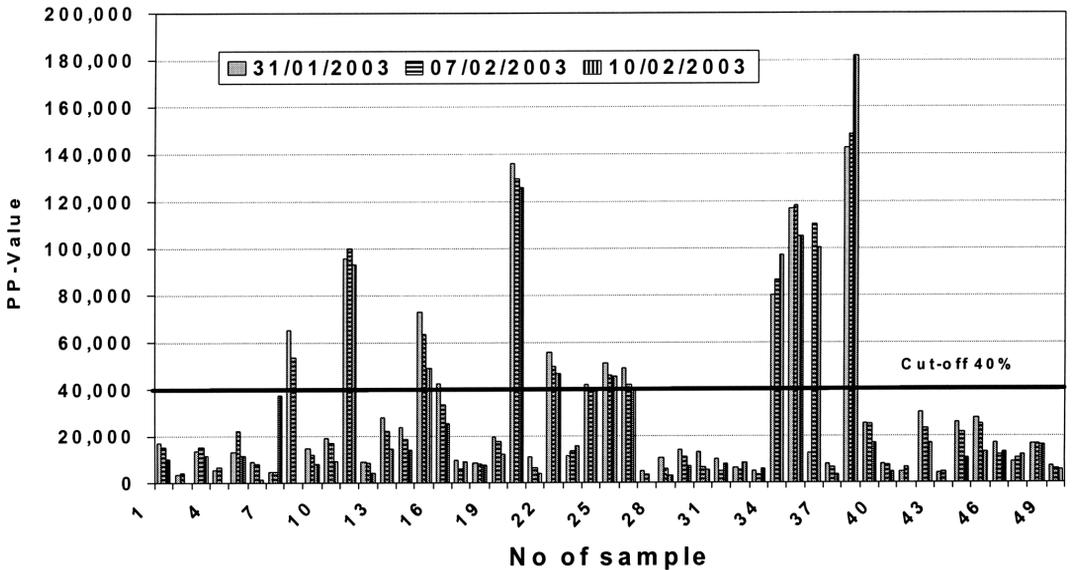
Graph 2: ELISA results of blood and serum samples taken from the same pigs at slaughterhouse 2



Consecutive blood sampling

The results show a very good correlation between blood samples taken up to 10 days apart. Some animals show a slight increase in PP-values whereas others show a slight decrease. However most of the samples taken at timepoint 1 and 10 days later have the same qualitative result in the ELISA and a very similar quantitative result.

Graph 3: ELISA-results of blood samples taken from the same pigs on various days.



Discussion

The results of this investigations show that meat juice and serum samples of the same animals can give almost identical ELISA-results. However correct sampling technique is an important factor.

The serum samples in slaughterhouse 2 show generally higher results than the meat juice samples of the same animals and in some cases different qualitative results are achieved. A conclusive explanation for this has not been found yet, investigations are ongoing.

In other cases meat juice samples show higher results than serum samples and this is usually explained with blood contamination of the meat juice sample.

A variety of factors can influence the quality of meat juice samples like sampling-location, sample size, contamination with blood and timing of sampling. Especially the timing might be critical as meat samples taken soon after the killing of the animal will contain more blood than samples taken a longer time after the killing-process, as more blood will have dripped out of the carcass by then.

Blood samples may be the more standardized samples. As the examination of blood samples taken on different days shows the results are not significantly influenced even if the samples are taken up to 10 days apart. Naturally samples that have a PP-value close to the cut-off level can produce qualitatively different results when the same animals are sampled a few days later. However given the fact that the Salmonella control programs are based on herd results rather than on results of single pigs this should not significantly alter the categorization of the whole herd.

Conclusions

The ELISA-Test "Enterisol® Salmonellen Diagnostikum" can deliver almost identical results in meat juice and serum samples from the same animals

Blood samples may be the better defined samples.

Blood samples taken in a period of up to 10 days will deliver quantitatively almost identical results.

PD 10

Randomly amplified polymorphic DNA (RAPD) typing of *Salmonella* Senftenberg in animal feed production.

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Summary: Randomly Amplified Polymorphic DNA (RAPD) was studied as genotyping method typing for a strain collection of *Salmonella*, belonging to the serotype Senftenberg. This collection consisted of 48 strains/isolates that were sampled in Sweden during 1995-96. In this study the aim was evaluate the usefulness of RAPD in terms of discriminatory power, reproducibility and typeability to compare it with other genotyping methods. By using *Taq* and *Tth* DNA polymerase in separate reactions the ability to increase discriminatory power and reproducibility was studied. When *Tth* DNA polymerase was used in the RAPD reaction brighter bands were obtained and the reproducibility was increased. The results suggest that RAPD can be used for rapid screening of a strain material and in combination with other more discriminating genotyping methods to more truly reflect the genetic diversity.

Key words: animal feed, DNA polymerase, genotyping, RAPD

Introduction: *Salmonella* spp. are some of the most serious contaminants of food products and thus is of major concern to the food industry. These bacteria are well-known agents for food-borne outbreaks of human gastroenteritis (salmonellosis) in Europe. *Salmonella* is transmitted to humans via animals infected by consuming contaminated feed (Hinton, 1988; Jones et al, 1982). The development of molecular markers as well as improved detection methods for DNA has been of great importance and will in the future help to prevent the spread of infections and provide data about the best choices for treatment (Ling et al, 1998). In this particular case the goal was to compare *S. Senftenberg* isolates from an animal feed factory in Sweden, where it had been established as a house flora, with other more or less related isolates. *S. Senftenberg* is considered to be more resistant towards acidification, heating, desiccation and irradiation compared to other serotypes (Liu et al, 1969), which makes the risks for sanitizing problems bigger. RAPD is both a less costly and more time-efficient method than several other genotyping methods, like ie Pulse-field gel electrophoresis (PFGE). One particular aim of this study was to see how well it could be discriminate between epidemiologically related strains at the subserotype level.

Material and methods: From the collection of strains of *S. Senftenberg* samples were streaked onto Tryptone Glucose Agar (TGE) plates, to check for their purity. One colony was transferred to buffered peptone water (BPW). DNA was subsequently isolated, using the Easy DNA™ Kit (Invitrogen). The DNA concentrations were measured using a fluorometer (Turner Designs). From each DNA preparation (1:10 dilution in ddH₂O) 5 ml was taken to RAPD analysis. The total volume was 25 ml in each reaction tube. The PCR reagents that were added to each sample contained 1 x PCR buffer for each enzyme, supplied with 1U of *Taq* or *Tth* DNA polymerase (Roche), 0.2 mM of each of the four nucleotides dATP, dCTP, dGTP and dTTP (Roche), 1.0 mM of primer S 1254, sequence (5' → 3'): CCG CAG CCA A (Scandinavian Gene Synthesis) and 2.5 mM of MgCl₂ (Roche). The temperature programme of the thermal cycler included: an initial denaturation step of five minutes at 94 °C, 45 cycles of denaturation (30 seconds at 94 °C), annealing (30 seconds at 30 or 36 °C) and extension (40 seconds at 72 °C) and a final extension step of seven minutes at 72 °C, followed by cooling to 4 °C. The amplification products were analysed by electrophoresis, using 1 % agarose gel. Cluster analysis by GelComparII was performed,

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using the unweighted pair group method with arithmetic averages (UPGMA) for the Dice coefficient (band based) and Pearson correlation (curve based) method, respectively. To illustrate the reproducibility of fingerprints from single strains, five duplicates (from different gels) were used in a cluster analysis, where the average similarity coefficient from duplicates was calculated (see Figure 1). Finally, a numerical index of discrimination (D) (Hunter & Gaston, 1988) was calculated for RAPD analysis.

Results: Cluster analysis by GelComparII gave in all 9 RAPD types with *Tth* DNA polymerase, identified with at a similarity index of >80 %. The average number of reproducible electrophoresis bands per strain was 12.0 for *Taq* and 12.8 for *Tth* DNA polymerase. No correlation was found between the DNA concentration of the sample preparations and the number of electrophoresis bands, both for the RAPD analysis with *Taq* and *Tth* DNA polymerase, respectively. In the calculation example of reproducibility for PCR duplicates (Figure 1) a similarity coefficient between 83 and 97 % was obtained (average 88 %) The index of discrimination was calculated for *Tth* DNA polymerase ($D = 0.614$). When both enzymes are used for this calculation, the index of discrimination will become higher.

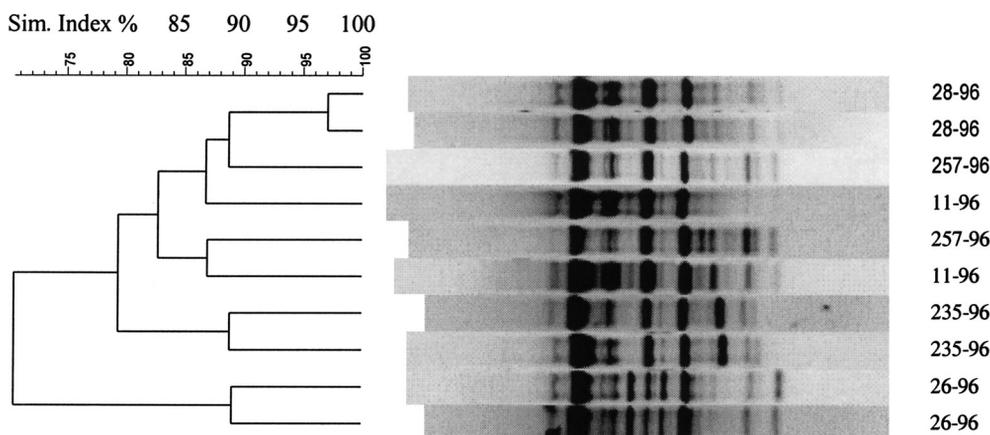


Figure 1. Cluster analysis of duplicate RAPD fingerprints from analyses with *Tth* DNA polymerase. The cluster analysis was performed with the curve-based clustering method in GelComparII in order to illustrate the reproducibility of the method. All pairs of duplicates were from different PCR reactions.

Discussion: The result from the analyses with *Tth* DNA polymerase was that they generally gave more distinct RAPD fingerprints than those made with *Taq* DNA

polymerase. The similarity level within the clusters in the dendrograms for *Tth* was generally higher than for *Taq* DNA polymerase, regardless of which clustering method that was used. In general it seemed that the use of *Tth* DNA polymerase increased stability of the PCR system. The RAPD fingerprints from the experiments showed that there are several demands that have to be met in order to make a correct statistical analysis by GelComparII. First the cluster analysis should preferably be based on a *limited* part of the gel. The part of the gels with the generally highest number of *discriminating bands* should be chosen, to make sure that the fingerprints are analysed in the most optimal way. To achieve the highest possible reproducibility the operations and equipment for RAPD analysis need to be well standardised. The results also showed that RAPD can be used for typing of strains within the serotype S.Senfthenberg.

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PD 11

SYBR Green Real-Time PCR for Salmonella detection in meat products

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Summary: The objective of this study was to develop a SYBR Green Real-Time PCR method for detecting salmonellae in meat samples. The study was conducted both on *S. Typhimurium* experimentally and naturally contaminated meat samples analyzed in parallel with the standard cultural method (ISO 6579/2001). After the pre-enrichment phase, a boiling DNA extraction procedure combined with SYBR-Green I Real Time PCR, using primers Styinva-JHO-2, was developed. The specificity of the reaction was confirmed by the Melting Temperature (T_m), which was consistently specific for the amplicon obtained (*S. Typhimurium* T_m=77.33±0.058). The standard curve constructed using the mean threshold cycle (C_t) and various concentrations of *S. Typhimurium* (ranging from 10³ to 10⁸ cfu/ml) showed a good linearity (R²=0.9767) and a sensitivity limit of less than 10³ cfu/ml. The comparison with the ISO method confirmed the effectiveness of the proposed method.

Keywords: rapid methods, pathogens detection, food.

Introduction: Salmonella continues to be one of the major causes of food poisoning in the western world. Different methods have been developed in order to reduce the time for the detection of the salmonella from food, since the ISO standard cultural method requires up of five days. Many of the PCR assays employ either visual scoring of ethidium bromide-stained agarose gels or post-PCR hybridisation-capture methods that are labour intensive, time consuming and difficult to automate. Recently, the use of double stranded DNA binding dye SYBR Green I for the detection of PCR product allows an early and simple approach to the Real-Time PCR and require less knowledge than classic Real-time PCR using fluorogenic oligoprobes (Hoorfar et al., 2000). The objective of the present study was to develop a SYBR Green I Real-Time PCR method for the detection of salmonellae in meat products. The experiments were conducted on *S. Typhimurium* experimentally and naturally contaminated meat samples and the results were compared to the standard cultural method.

Materials and methods: a sample was experimentally contaminated as follows: twenty-five grams of pork meat homogenized with 225 ml of buffered peptone water in a Stomacher were incubated at 37 °C for 24 h. The pre-enrichment broth was then divided into three aliquots: the first aliquot was used to confirm the absence of salmonellae by means of standard cultural method (ISO 6579/2001);

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the second aliquot was spiked with an appropriate quantity of *S. Typhimurium* suspension to obtain a final concentration of 10^7 cfu/ml; the third aliquot was used as negative control.

Thirty meat samples purchased from local retail outlets were analyzed both with the standard cultural method and with the SYBR Green I Real-time PCR.

The extraction and purification of DNA was performed by boiling according to the procedure previously described (De Medici et al., 2003). Five μ l of the supernatant, used as DNA template.

The PCR was performed using the Styinva-JHO-2 primers (Hoorfar et al., 2000) at the concentration of 50nM.

The amplification reactions were performed in a total volume of 50 μ l with an ABI Prism 7700 sequence detector 96-well micro-well plates. In each well, we placed 5 μ l of purified DNA, 25 μ l SYBR Green I PCR Master Mix (Applied Biosystems), 50nM primer Styinva-1, 50nM primer Styinva-2, and, to reach a total volume of 50 μ l per well, DNase-RNase-free distilled water. The reaction was run online at 50°C for 2 min and 95°C for 10 min, followed by 35 cycles at 95°C for 1 min, 55°C for 1 min and 72°C for 60 sec, with an extension phase of 1 cycle at 95°C for 1 min, 60°C for 1 min, and 95°C for 1 min (ramp time 19.59 min).

The results were visualized using the software Sequence detector 1.7 provided with the ABI Prism 7700 system. The specificity of the reaction is given by the T_m of the amplification products immediately after the last reaction cycle.

Standard curve: it was obtained from the C_T values of the meat sample in pre-enrichment broth after incubation at 37°C for 24 h, supplemented with tapering concentrations of *S. Typhimurium* (10^8 , 10^7 , 10^6 , 10^5 , 10^4 , and 10^3 cfu/ml). One non-spiked sample was used as a negative control.

Standard cultural method: it was performed according ISO 6579/2001 method. Suspected salmonellae colonies were identified using API 20E system and commercially available specific antisera.

Results: the mean peak T_m obtained with the curves specific for *S. Typhimurium* obtained from various experiments was 77.33 ± 0.058 . Of the 30 samples considered, 11 were found to be positive when analyzed both using SYBR-Green I Real-Time PCR (CT value ranging from 16.04 ± 0.221 to 18.892 ± 0.183) and the standard cultural method (table 1).

Table 1. Serotypes identified by the standard cultural method and T_m values obtained by SYBR Green I real-time PCR

| Sample | ISO 6579/2001 Serotype | SYBR-Green I Real-Time PCR T_m (°C) |
|--------|------------------------|---------------------------------------|
| 1 | <i>S. Newrochelle</i> | 77.03 ± 0.231 |
| 2 | <i>S. Infantis</i> | 77.17 ± 0.008 |
| 3 | <i>S. Typhimurium</i> | 77.27 ± 0.031 |
| 4 | <i>S. Enteritidis</i> | 77.25 ± 0.016 |
| 5 | <i>S. London</i> | 77.30 ± 0.348 |
| 6 | <i>S. London</i> | 77.20 ± 0.000 |
| 7 | <i>S. Infantis</i> | 77.27 ± 0.153 |
| 8 | <i>S. Enteritidis</i> | 77.28 ± 0.021 |
| 9 | <i>S. Typhimurium</i> | 77.30 ± 0.081 |
| 10 | <i>S. Newrochelle</i> | 77.13 ± 0.231 |
| 11 | <i>S. Typhimurium</i> | 77.36 ± 0.062 |

The negative samples used as control did not show peaks with SYBR-Green I Real-Time PCR. The standard curve showed a good linearity of response ($R^2=0.9767$). The sensitivity limit of the reaction was less than 10^3 cfu/ml.

Discussion: the proposed method seems to be effective, rapid and reproducible, the comparison with the standard method showed that neither false-positive nor false-negative results were obtained. The specificity of the reaction was confirmed by the determination of the T_m , specific for the amplicon obtained, that allows to eliminate the phase of electrophoresis, which is time-consuming and requires the use of ethidium bromide, a potent mutagenic agent, that is not suitable for routine use.

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PD 12

Development of an ELISA test for *Salmonella* serological monitoring in Brazil

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Summary: EMBRAPA/CNPAS developed an ELISA test based on LPS antigens from *Salmonella* Typhimurium. After the optimal dilution determination of the test components, four sera were chosen as controls. The interplate variation was controlled by a coefficient correlation between standard and daily curves of control sera and the coefficient variation of sample sera triplicates. The cut-off was determined by a dispersion analysis in a nursery piglet population proved to be salmonellae negative. The test performance was evaluated in experimentally and naturally *S. Typhimurium* infected pigs and in animals vaccinated with other *Salmonella* serovars. The seroconversion was observed after two weeks post inoculation in experimentally infected and vaccinated animals. In naturally infected animals, which were sampled twice during the finishing period, at the first sampling 75 % of pigs were eliminating salmonellae in feces and 25 % were positive in the ELISA. At the second sampling 76.9 % became serologically positive. These results suggest that the developed test can be used for *Salmonella* Typhimurium monitoring programs in swine.

Keywords: Lipopolysaccharides, *S. Typhimurium*, swine, serology.

Introduction: Previous studies conducted in southern Brazil indicated a wide dissemination of salmonellae infection in swine herds (Bessa et al. 2001, Kich et al. 2001). The reduction in the number of carrier pigs at slaughter is one of the most important measures for pork contamination control. Most countries started intensive programs of *Salmonella* control on farms based on serological monitoring (Nielsen et al., 2001). In southern Brazil serovars Typhimurium, Agona, Derby, Bredney and Panama have proved to be the most prevalent in carrier pigs sampled at slaughter (Bessa et al., 2001). As these serovars have at least two common LPS antigens with Typhimurium, an ELISA test was developed based on LPS antigens from *S. Typhimurium*.

Material and Methods: Phenolic extraction of LPS from *Salmonella* Typhimurium was done as described previously (Vidal et al. 1999). Optimal dilution was determined for serum (1:400), antigen (1:2000) and conjugate (1:25,000). For control of intraplate and interplate variation reference sera, chosen from sera

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of SPF and inoculated pigs, were included on every plate. The mean OD's for these sera in 15 assays were 0.25, 0.245, 0.4385 and 1.0995. Sample OD's were transformed to calibrated OD, using a linear regression equation for reference OD's on the actual plate versus mean reference OD's. For an intraplate variation control, all sample sera were tested in triplicate and the acceptable variation coefficient among sera OD's was below 10%. The cut-off (OD 0.159) was calculated as the mean OD of a negative population plus four standard deviation. Orally infected pigs were submitted to the ELISA test, in order to observe the seroconversion. Three 95-days-old SPF pigs were inoculated orally with 3.5×10^8 cfu of *Salmonella* Typhimurium and two animals were kept as sentinels. Blood was collected weekly from all animals during a period of 42 days. Furthermore, a field study was conducted including 56 pigs tested at the early growing phase and at slaughter. Blood and fecal samples were taken from all pigs. Finally, groups of five pigs (aged 55 days) were vaccinated with bacterins produced individually with serovars Typhimurium, Agona, Derby, Bredney and Panama. Four non - inoculated pigs were kept as negative controls.

Results: Seroconversion in inoculated and vaccinated pigs was observed around two weeks after the exposure. Sentinels became positive between the third and fourth week after the challenge. Higher OD values were observed in inoculated pigs than in sentinels. In the field trial, 75 % (42/56) of the growers were shedding *Salmonella* Typhimurium in feces and 25 % (14/56) were serologically positive. At slaughter the serological prevalence increased to 76.9 % in the same group of animals.

Discussion: Several studies described salmonellae mix ELISA tests to monitor infection on farms (Nielsen et al.,1995; Vidal et al, 1999; Proux et al., 2000). The present study showed that natural and experimental infection by *Salmonella* Typhimurium can be detected using the developed ELISA test. Furthermore, the test proved to be able to detect also the most prevalent serovars found by Bessa et al. (2001) in southern Brazil. More studies have to be conducted in order to compare the performance of the developed ELISA test with salmonellae mix ELISA tests currently in use.

Conclusions: The developed ELISA test detects antibodies against salmonellae serovars prevalent in southern Brazil and can be adopted for screen pigs for serological evidence of infection.

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ANTI-SALMONELLA LACTIC ACID BACTERIA FROM PORCINE INTESTINAL SOURCES

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Summary: The aim of this study was to isolate lactic acid bacteria (LAB) with anti-*Salmonella* activity from the porcine gastrointestinal tract (GIT) and to characterise these for potentially probiotic properties using *in vitro* assays. Porcine caecal and faecal samples were screened for the presence of anti-*Salmonella* LAB; the ten most promising isolates belonged to the genera *Lactobacillus* and *Pediococcus*. The LAB exhibited large variation in their ability to survive in simulated gastric juice at pH 1.85. While *Lactobacillus acidophilus* species survived at up to 80% for 30 min, *Lb. pentosus* species declined to less than 0.001%. All isolates tolerated porcine bile at a concentration of 0.3%, with some capable of growth in the presence of up to 5% bile. The ability of the LAB isolates to prevent *Salmonella* invasion of intestinal epithelial cells varied, with reductions of 55% (*Lb. acidophilus* spp.) to 82% (*Lb. salivarius* spp.) observed. The data demonstrates that some porcine intestinal LAB isolates may offer potential as probiotics for the reduction of *Salmonella* carriage in pigs.

Keywords: probiotic, pigs, *Salmonella*, *Lactobacillus*, pathogen

Introduction: Probiotics offer potential to reduce intestinal *Salmonella* carriage in pigs. Potentially probiotic bacterial cultures must possess certain properties if they are to function effectively in the intestine. Of prime importance amongst these is the ability to survive passage through the GIT; consequently, acid and bile tolerance are important criteria, as well as an ability to exert the probiotic effect at the target site. In this study, LAB isolates were characterized in relation to potentially probiotic traits using a range of *in vitro* procedures.

Materials and Methods: LAB were initially isolated from porcine faecal and caecal samples by growth in Brain Heart Infusion (BHI) broth and both liquid and solid de Man Rogosa Sharpe (MRS) medium. Isolates with anti-*Salmonella* activity were identified by spot plating onto MRS agar and, following overnight incubation at 37°C, overlaying with a lawn of *Salmonella* Typhimurium in BHI agar; these isolates were then stocked. The ten isolates with greatest anti-*Salmonella* activity were identified and further characterized. Tolerance to gastric juice was examined by suspending overnight cultures in synthetic gastric juice adjusted to pH 1.85 and incubating at 37°C. After 30 min, viability was measured by plating on MRS agar. The synthetic gastric juice solution consisted of 3.5 g/l D-glucose, 2.05 g/l NaCl, 0.6 g/l KH₂PO₄, 0.11 g/l CaCl₂, 0.37 g/l KCl, 0.05 g/l porcine bile, 0.1g/l lysozyme and 13.3 mg/l pepsin. Tolerance to bile was assayed by streaking cultures onto MRS plates containing porcine bile at concentrations of 0 – 5% (w/v) and examining the plates for growth after 72 h. The ability of the isolates to prevent invasion of HT-29 human intestinal epithelial cells by *Salmonella* was investigated. Volumes (1 ml) of overnight isolate resuspended in Dulbecco's Modified Eagle Medium (DMEM) were added to wells of washed confluent HT-29 cells in six-well Corning tissue culture plates and incubated at 37°C for 1 h. One ml volumes of similarly-treated *Salmonella* Typhimurium DT104 cells, adjusted to give a multiplicity of infection ratio of 100:1, were then added and incubation continued for another hour. Bacterial cells were then removed from wells and DMEM containing 100 mg/ml gentamicin was added and plates were incubated for a further hour. The monolayers were then washed several times with phosphate buffered saline (PBS) and lysed; the resultant lysate was serially diluted and

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Salmonella counts were obtained by plating on tryptic soya agar (TSA) plates. Control wells were treated similarly except that DMEM was added instead of isolate suspension.

Results: Initial examination of approximately 6000 colonies resulted in 173 isolates giving zones of inhibition with a radius of greater than 5 mm. The ten isolates exhibiting the greatest anti-*Salmonella* activity in these assays were identified by 16S rRNA sequencing (Table 1). Survival of these isolates in synthetic gastric juice (pH 1.85) varied from 0.004% to 79.29% after 30 min exposure (Table 1). *Lb. acidophilus* 4 survived better than any other isolate; indeed its survival (79.29%) was approximately 200 times greater than that of *Lb. salivarius* 18, the next best surviving isolate. *Lb. salivarius* strains performed well, with isolates DPC6005, 7.3 and 18 surviving relatively well. All isolates tested grew in conditions of 0.3% bile, while the maximum level tolerated varied from this concentration up to 5% (Table 1). *Lb. acidophilus* 4 and *Lb. pentosus* DPC6004 both grew in the presence of 5% bile. Several isolates, particularly *Lb. acidophilus* 4, were associated with a cloudy zone of precipitation in the medium which may be indicative of bile salt hydrolase production (Dashkevicz and Feighner, 1989). All isolates tested adhered to HT-29 intestinal cells (data not shown). When examined for their ability to prevent *Salmonella* invasion of HT-29 cells where the control represented 100% *Salmonella* invasion, levels of invasion in the presence of isolates varied from 18.57% to 46.02% (Table 1). The greatest reduction in invasion was associated with the *Lb. salivarius* strains DPC6005, 7.3 and M7.2. Isolates *Lb. acidophilus* 4 and *Lb. pentosus* DPC6004 were the least effective in inhibiting *in vitro* *Salmonella* invasion (Table 1).

Table 1. Probiotic properties of porcine intestinal isolates

| Isolate | <i>Salmonella</i> inhibition ¹ | Gastric juice survival ² | Bile Tolerance ³ | Invasion ⁴ |
|-------------------------------|---|-------------------------------------|-----------------------------|-----------------------|
| <i>Lb. murinus</i> DPC6002 | 6.3 | 0.0029 ^a | 0.3 | 21.96 |
| <i>Lb. murinus</i> DPC6003 | 6 | 0.0021 ^a | 0.3 | 21.76 |
| <i>Lb. pentosus</i> DPC6004 | 6.7 | 0.0024 ^a | 5.0 | 44.17 |
| <i>Lb. salivarius</i> DPC6005 | 9 | 0.0391 ^b | 0.5 | 18.57 |
| <i>P. pentosaceus</i> DPC6006 | 7.3 | 0.0026 ^a | 2.0 | 26.87 |
| <i>Lb. acidophilus</i> 4 | 6 | 79.29 ^c | 5.0 | 46.02 |
| <i>Lb. agilis</i> 13 | 9 | 0.0004 ^a | 0.3 | 36.84 |
| <i>Lb. salivarius</i> 18 | 6.8 | 0.3732 ^d | 0.3 | 30.47 |
| <i>Lb. salivarius</i> 7.3 | 7 | 0.0293 ^b | 0.3 | 20.89 |
| <i>Lb. salivarius</i> M7.2 | 8.3 | 0.0028 ^a | 0.3 | 21.33 |
| Control | N/A | N/A | N/A | 100 |

¹Radius (mm) of zones of inhibition produced by isolates in plate assays with *Salmonella* Typhimurium.

²Results are expressed as percentage survival after 30 minutes in synthetic juice, pH 1.85. Different superscripts represent values statistically different from each other ($P < 0.05$).

³Values represent the maximum concentration of bile (% w/v) at which growth was observed on MRS plates.

⁴Inhibition by porcine isolates of *Salmonella* invasion of HT-29 intestinal epithelial cells. Results are expressed as percentage invasion in the presence of cultures (control = 100% *Salmonella* invasion).

Discussion: In this study, ten porcine bacterial intestinal isolates were selected on the basis of anti-*Salmonella* activity and investigated for their probiotic potential. The first major hurdle bacteria must overcome in the GIT is the low pH of the gastric contents. In simulated gastric transit studies using synthetic gastric juice at pH 1.85, *Lb. acidophilus* 4 exhibited the strongest survival after 30 min; this is in agreement with previous reports linking *Lb. acidophilus* with good survival under acidic conditions (Marteau *et al.*, 1997). This isolate also survived well in the presence of bile, a major impediment to bacterial survival in the small intestine; however, it was also apparently a producer of bile salt hydrolase, which has been linked to colorectal cancer (Nagengast *et al.*, 1995). Levels of *Salmonella* invasion of intestinal epithelial cells in the presence of isolates spanned a relatively narrow range,

varying from 18.57% to 46.02%. The greatest reduction in invasion (approximately 80%) was associated with isolates of *Lb. salivarius* and *Lb. murinus*. Interestingly, *Lb. acidophilus* 4, which performed well in the previous assays, was the least effective at preventing *Salmonella* invasion.

Conclusions: The results of the present study support the potential use of some porcine intestinal LAB as probiotics for the reduction of *Salmonella* carriage in the pig GIT.

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PI 01

Effects of commercial feed additives on Porcine intestinal microflora

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Summary: The objective of this study was to assess the effects of commercially available feed additives on the gut microflora of finishing pigs. Pigs received either a barley-based control diet, or an experimental diet supplemented with mannanoligosaccharide (BioMOS™), fumaric acid, or a commercially available acid/salt mixture (Bact-A-Cid™) for four weeks prior to slaughter. Dietary supplementation with fumaric acid (20 g/kg) resulted in the greatest effects on gut microflora composition. Following 28 days of treatment, faecal coliforms and lactobacilli numbers were reduced in the fumaric acid-fed animals (P<0.05). In addition, there was a ten-fold reduction in lactobacilli in the caecum and colon due to fumaric acid treatment (P<0.05). The data indicate that supplementation with fumaric acid caused a desirable change in coliform numbers. However, given that *Lactobacillus* are considered beneficial microorganisms in the mammalian intestine, the reduction in lactobacilli counts as a result of fumaric acid supplementation warrants further investigation.

Keywords: pigs, gastrointestinal tract, bacteria, acid, mannanoligosaccharide

Introduction: A possible means of controlling carcass contamination with salmonellae and reducing consequent public health concerns, is to reduce intestinal carriage of the organism in finishing pigs by incorporation of additives into the feed. Reduced numbers of pathogenic bacteria in weanling pigs supplemented with organic acids and salts (Canibe *et al.*, 2001; Tsiloyannis *et al.*, 2001) has been attributed to reduced pH and dissociation of acid molecules in the cell cytoplasm. Dietary supplementation with mannanoligosaccharide (MOS) or D-mannose has been shown to effectively reduce colonisation by *Salmonella* Typhimurium in broiler chicks (Spring *et al.*, 2000), and *Escherichia coli* K88 in piglets (White *et al.*, 2002) by reduced adherence of pathogenic bacteria to epithelial cells.

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However, relatively few studies have been performed using acidifiers or MOS in pigs during the latter stages of production. The objective of this study was to compare the effects of such feed additives on the faecal and gastrointestinal microbiology of finishing pigs.

Materials and Methods: Female pigs were selected at 75 kg, blocked on weight, and assigned to one of four treatments in a randomised complete block design as follows: T1. Control diet (800 g/kg barley), T2. Control diet + 1.5 g/kg BioMOS[®] (Alltech), T3. Control diet + 3 g/kg Bact-A-Cid[®] (Agil Products) and T4. Control diet + 20 g/kg fumaric acid. There were a total of eight pigs per treatment. Faecal samples were taken prior to treatment and after 7, 14, 21 and 28 days on trial. The pH values of the samples were recorded. Samples were then diluted 1:10 in maximum recovery diluent (MRD) containing glycerol (40 % (v/v)), and stored at -20 AC pending microbiological analysis. At the end of the four-week feeding period, pigs were slaughtered, and gastrointestinal contents were sampled from the pyloric region of the lower ileum, caecum, and ascending colon. Intestinal samples were treated in the same manner as faeces. Microbiological analysis was conducted on faecal and intestinal samples as follows. Coliforms were enumerated on violet red bile agar following incubation at 37 AC for 24 h. Bifidobacteria and lactobacilli were enumerated on de Man, Rogosa & Sharpe agar containing 0.05 % (w/v) cysteine hydrochloride and mupirocin (200 mg/ml) and *Lactobacillus* selective agar, respectively, following anaerobic incubation at 37 AC for 72 h. The General Linear Model procedure of SAS was used to analyse the data, and Duncan's Multiple Range Test was used for separation of means.

Results: Results for microbiological analysis of faecal and intestinal samples are shown in Table 1. Faecal coliforms were significantly reduced ($P<0.05$) by fumaric acid supplementation after 28 days compared with control animals. A significant reduction ($P<0.05$) in faecal lactobacilli was also seen with fumaric acid after 7 days (data not shown), and this effect remained throughout the trial. At slaughter there was a numerical trend towards lower coliform numbers in the caecum and colon as a result of fumaric acid treatment compared with control animals ($P>0.05$). In addition, there was a 10-fold reduction in *Lactobacillus* counts in the caecum and colon of fumaric acid-fed animals compared with all other treatments ($P<0.05$). Bifidobacteria in intestinal samples remained largely unchanged by treatment. However, a reduction in numbers was observed in the ileum with Bact-A-Cid[®] supplementation ($P<0.05$) (data not shown). No differences in pH of intestinal or faecal samples were found (data not shown).

Table 1. Mean counts of intestinal and faecal microflora (Log cfu/g) in pigs following administration of a control diet or treatment with BioMOS[®], Bact-A-Cid[®], or fumaric acid

| Microbiological analyses | Control diet | BioMOS [™] (1.5 g/kg) | Bact-A-Cid [™] (3.0 g/kg) | Fumaric acid (20 g/kg) |
|--------------------------|-------------------|-----------------------------------|---------------------------------------|---------------------------|
| Coliform | | | | |
| Faeces day 0 | 6.40 | 5.97 | 6.35 | 6.33 |
| Faeces day 28 | 6.72 ^a | 6.29 ^{ab} | 5.97 ^{ab} | 5.58 ^b |
| Caecum | 7.61 | 7.11 | 7.07 | 6.92 |
| Colon | 7.22 | 6.78 | 7.03 | 6.57 |
| Lactobacilli | | | | |
| Faeces day 0 | 8.96 | 9.19 | 9.02 | 9.09 |
| Faeces day 28 | 8.95 ^a | 9.07 ^a | 9.09 ^a | 8.14 ^b |
| Caecum | 8.34 ^a | 8.33 ^a | 8.68 ^a | 7.61 ^b |
| Colon | 8.83 ^a | 8.85 ^a | 8.99 ^a | 7.67 ^b |
| Bifidobacteria | | | | |
| Faeces day 0 | 4.03 | 4.72 | 4.95 | 4.40 |
| Faeces day 28 | 4.28 | 5.03 | 4.98 | 5.02 |
| Caecum | 3.95 | 4.71 | 4.55 | 4.99 |
| Colon | 4.34 | 4.98 | 4.77 | 4.95 |

^{a,b} Means within the same row showing different superscripts are significantly different ($P<0.05$)

Discussion: Our study found that MOS supplementation of finisher pig diets resulted in no significant alterations in faecal or gastrointestinal pH or microbial populations compared with other treatments

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($P > 0.05$). This agrees with previous results obtained on feeding MOS to weaner pigs (Mathew *et al.*, 1998). It is, however, in contrast to studies performed by White *et al.* (2002) who found that piglets supplemented with brewers dried yeast as a source of MOS had lower numbers of total coliforms. MOS has also been shown to act as effective antimicrobial agent in experimental infection studies, where animals were challenged with *S. Typhimurium*, a species known to be mannose-sensitive (Oyofe *et al.*, 1989; Spring *et al.*, 2000). MOS affects gut microflora through the adsorption of bacteria (Spring *et al.* 2000), and it is likely that the lack of effect of MOS supplementation observed in the present study may be due to a lack of mannose-specific receptors on the cell surface of the species examined (lactobacilli, bifidobacteria, coliform). However, further studies are required to establish the effectiveness of MOS supplementation in reducing carriage of food-borne pathogens in finishing pigs. Our study found that faecal, but not intestinal coliforms were reduced as a result of fumaric acid treatment ($P < 0.05$). Apart from the effects on health (i.e. reduced diarrhoea), the inclusion of acids and/or salts to the feed/water of finishing pigs may provide a means of reducing carcass contamination at slaughter by reducing levels of intestinal carriage of enteropathogens (van der Wolf *et al.*, 2001). However, the reduction in coliform observed in the present study was also accompanied by a decrease in both faecal and intestinal lactobacilli ($P < 0.05$). Lactobacilli are considered a beneficial intestinal bacterial species, and so the implications of this finding are unknown. It has previously been suggested that this reduction in lactic acid bacteria is due to negative effects on lactic acid fermentation by fumaric acid. An alternative acid such as lactic acid may, therefore be more suitable for the enhancement of *Lactobacillus* populations (van Winsen *et al.*, 2001).

Conclusions: Fumaric acid supplementation resulted in the greatest modifications in gut microflora, including reduced coliform and lactobacilli numbers. However, these reductions in microbial populations were mainly observed for *Lactobacillus* spp., bacteria considered potentially beneficial to the animal. Apart from fumaric acid, little evidence of a significant effect on intestinal pH or gut microflora of finishing pigs was observed when diets were supplemented with the additives used. This is likely to be due, in part, to the age and health status of the pigs used in the study.

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Elimination of *Listeria* from a sausage batter by HHP treatment

PI 02

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Summary: Samples of uninoculated batter of a typical Hungarian fermented pork sausage with or without regular NaCl and nitrite additive, and similar samples but heavily inoculated with *Listeria monocytogenes* have been treated by high hydrostatic pressure (HHP) of 600 MPa for 20 min and reduction of the viable cell count of the test organism was selectively estimated. The HHP treatment caused 5 log-cycles reduction of *Listeria* count in the salt-free batter and around 4 log-cycles in the salt-containing samples. Discolouration of the batter as an effect of the high pressure treatment was observed. It was less pronounced in the nitrite-containing samples. The HHP reduced softness of the batter and changed drastically the pattern of its DSC thermogram pointing for significant protein denaturation.

Keywords: Decontamination, *Listeria monocytogenes*, high pressure

Introduction: Due to its ability to inactivate vegetative bacterial cells, high hydrostatic pressure above 200 MPa can be one of the alternative non-thermal pasteurization techniques. *Listeria monocytogenes* as an environmental contaminant may contaminate meat trims and it is capable to growth at temperatures of refrigeration and in high-salt environment. Because of the high mortality rate associated with listeriosis, *L. monocytogenes* is given zero tolerance in ready-to-eat meat products in the United States (USDA, 1989). The aim of our studies was to investigate the microbiological efficacy of high hydrostatic pressure to control *L. monocytogenes* in raw batter of a typical Hungarian fermented pork sausage. Studies were performed also to clarify the effects of the regular sodium chloride additive and its curing mixture with nitrite on the high pressure induced changes.

Materials and methods: Pork sausage batter of typical composition (Krommer et al., 2001) has been prepared at the National Meat Research Institute, Budapest. Experimental samples of non-inoculated batter and those, which have been heavily inoculated with a strain of *L. monocytogenes* 4ab before addition of the lactic starter culture, respectively, were divided into small plastic pouches and after sealing under vacuum have been subjected to high pressure treatment of 600 MPa for 20 min in a Stansted "FoodLab 900" type equipment while maintaining them at about room temperature as described by Hassan and others (2002). Analyses were performed immediately after the treatments. Total aerobic viable cell counts (TVC) and *Listeria* counts in inoculated samples were estimated in duplicate samples using plating in Oxford agar with *Listeria* selective supplement. Surface colour by a Minolta tristimulus colorimeter and texture by an SMS TAXT2i type texture analyser were estimated on uninoculated samples. Differential scanning calorimetry (DSC) with a SETARAM "MicroDSC III" microcalorimeter was used to characterize the extent of protein denaturation in HHP treated samples by observing changes in the heat denaturation pattern between 40 and 90 °C.

Results: Uninoculated samples possessed 7.10^5 TVC/g including 2.10^2 bacterial spores/g. The *Listeria* count of the inoculated batter amounted to 5.10^6 cfu/g. The HHP caused somewhat more than 3 log-cycles reduction in the TVC and less than one log reduction in the count of bacterial spores. The *Listeria* count decreased by 5 log-cycles in the salt-free batter, and around 4 log cycles in the salt-containing samples. HHP-treated samples showed increased lightness values and decreased redness values. The yellowness values increased by the pressurization in the salt-free samples but decreased in the

salt-containing ones. The discolouration was less pronounced in the nitrite-containing samples than in the nitrite-less samples. Compressing cylindrical samples of pressurized sausage batter by the texture analyser to their half-height required approx. 26 % more power in the salt-free samples, approx. 50 % more in the salt containing ones, and 58 % more in those containing both salts than in case of the respective non-pressurized samples. Previously, we have observed reduced softness with correspondingly increased water binding capacity when studied high pressure-induced effects on minced beef (Hassan et al., 2002). This phenomenon was in relation with non-thermal denaturation/coagulation of proteins. Since differential scanning calorimetry is proved to be an effective method to monitor thermal behaviour of proteins related to previous structural changes of meat (Findlay and Barbut, 1990), we have investigated DSC thermograms of untreated and HHP-treated batters. In the thermogram of salt-free, non-pressurized sample a small endothermic peak appeared between 50 and 55 °C, which could be attributed to the heat denaturation of myosin, and myosin subunits, followed by a composite transition between 57 and 68 °C, showing a distinct large peak and a shoulder which can be considered as heat denaturation of connective tissue (collagen and other stromal proteins) as well as those of sarcoplasmic proteins and the myoglobin. An other distinct peak between 73 and 77 °C was related to the heat denaturation of actin, actinins and troponins (Findlay and Barbut, 1990). The DSC thermograms of HHP-treated samples showed a less complex profile both as the number of heat denaturation endotherms and the enthalpy changes are concerned. These observation showed that most of the proteins of the high-pressure treated samples were in a denatured state in the pressurized batter, except the pressure-resistant collagen. The DSC thermograms of the salt-containing samples showed only one large endotherm at about 65 °C in the unpressurized state, and a greatly diminished one at slightly lower temperature in the pressurized samples.

Conclusions: Our results show that high hydrostatic pressure processing was effective in diminishing considerably *Listeria monocytogenes* inoculated into the raw sausage batter, but it caused some changes in its appearance, texture and protein structure. Discolouration and coagulation of muscle tissues due to HHP are, however, well known (Chefftel and Culioli, 1997). On this basis, further studies are initiated to monitor the fate of surviving microorganisms during fermentation and to assess the quality of the ripened sausage prepared from HHP-pasteurized batter, and to investigate the high pressure effects on electrophoretic and immunological cross-reactivity of its proteins (Hajós et al., 2003).

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FAILURE TO PROVE THE EFFECT OF FEEDING ON EXPERIMENTAL SALMONELLA TYPHIMURIUM INFECTION IN PIGS

PI 03

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Keywords: Feeding strategy, organic production, experimental infection

Summary: The hypothesis of the present investigations was that organic feeding with a high level of protein might increase the risk of development of infection with *S. Typhimurium* compared to conventional feed. Also feeding with pelleted feed might increase the risk compared to feeding with meal feed. An experimental infection study with *S. Typhimurium* including a total of 60 pigs in 4 feeding groups: organic pelleted feed, organic meal, conventional pelleted feed and conventional meal, was performed. Pigs were followed by microbiological examination of faecal samples and serological analysis of blood in 8 weeks. In the experimental model applied, it was not possible to show any significant difference in the establishment and course of infection with *S. Typhimurium* in relation to the feeding strategy. The variation of the infection among the individual pigs included in the study was very high which may cover a possible effect of the different feedings. This high degree of variation may be taken into account when future experimental investigations have to be planned.

Materials and Methods: An experimental study including a total of 60 salmonella negative pigs was performed (3 groups of 5 pigs of each of 4 feeding groups). The pigs in each of the feeding groups were fed with 1) organic pelleted feed, 2) organic meal, 3) conventional pelleted feed and 4) conventional meal. Organic feed had a higher crude fiber content compared to the conventional feed (8% vs. 3,7%) and a more wide panel of protein sources (soya beans, rape, peas and lupin vs. soya and synthetic aminoacids).

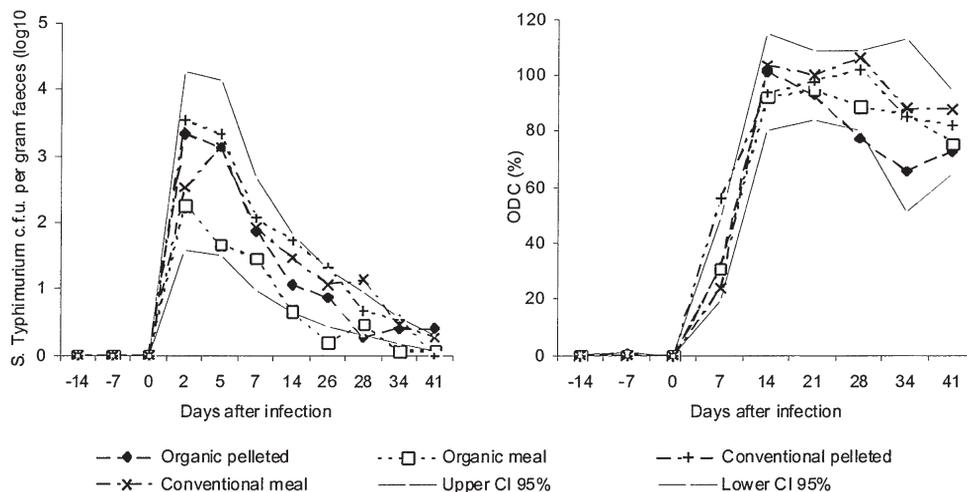
From the start and throughout each of the experiments, pigs were given the different types of feed and water *ad libitum*. After 14 days all pigs were infected orally by use of a stomach tube with approximately 10^9 c.f.u. of a rifampicin resistant strain of *S. Typhimurium* DT12 (Nielsen et al., 1995). Throughout the study faecal material and blood samples were collected once a week in 8 weeks. Before challenge faecal samples was analysed qualitatively for *Salmonella* whereas after challenge, excretion of the challenge strain was quantified by serial 10-fold dilution of the unincubated preenrichment buffers and subsequently analysis of each of the dilutions as described previously (Baggesen et al., 1999). Agglutination of the O5-factor and identification of resistance against rifampicin verified isolated strains as the challenge strains. The excretion of *S. Typhimurium* was stated as log₁₀ c.f.u per gram faeces. In addition, the faecal samples were examined by conventional bacteriological methods determining the level of *Enterobacteriaceae* (NMKL 144, 1992), *Enterococci* (NMKL 68, 1992) and *Lactobacillus* (NMKL 140, 1991). Blood samples were examined for the occurrence of salmonella antibodies by mix-ELISA covering O-antigens 4,5,6,7 and 12 (Nielsen et al., 1995) stated as OCD%. 95% confidence intervals for the mean salmonella count and the mean antibody OCD% was estimated as ± 2 (s.d. of the log₁₀(c.f.u) / \sqrt{n}) and ± 2 (s.d. of the OCD% / \sqrt{n}) respectively, by treatment group and by sampling day. With n=15 this equals the standard CI 95% for each treatment group average. Under the assumption of no treatment effect, the difference between the average of the four treatments and each treatment group average will have a CI 95% with a length of $\sqrt{3}/2$ times this quantity. Where samples were lost and sample sizes therefore less than 15 per treatment, CI 95% were adjusted accordingly.

Results: The salmonella infection was established in all pigs resulting in mean excreting of 10^2 to 10^4 c.f.u. *S. Typhimurium* per gram faeces 2 days after challenge followed by a decline in the excretion in the following weeks until the end of the experiments. Each of the infected pigs reacted at the infection by production of specific antibodies against *Salmonella*. The confidence limits were wide reflecting a large variation in the salmonella excretion (fig 1a) as well as in the antibody level (fig 1b) between individuals within each treatment group. No significant difference in the level of salmonella excretion (fig. 1a) or antibody titer (fig. 1b) between the four treatment groups could be detected. Neither in relation to the other bacteria investigated any differences between treatment groups could be detected.

Discussion and conclusions: Several epidemiological investigations have shown the significance of feeding strategy on the risk of salmonella infection in finisher pigs. Feeding with non-heat treated and optional also coarse-grained feed has a protective effect on prevalence of salmonella. In addition, the level of protein in the feed has shown to have a relation to development of diarrhoea and as a consequence to the possibility of establishment of salmonella infection.

In the experimental model applied, it was not possible to show any significant difference in the establishment and course of infection with *S. Typhimurium* in relation to the feeding strategy. The failure to demonstrate any effects of the different feeding strategy may reflect that there in fact are no effects of the feed applied or that the effects are so limited that they are concealed by the wide variation of the infection among the individual pigs included.

As salmonella infection in pigs is multifactorial, it has been involved with difficulties to establish a stable experimental model, which result in high degree of variation in the infection output. This variation shall be taken into account when future experimental investigations are designed.



Figur 1 Faecal excretion of *S. Typhimurium* (challenge strain) (a) and development of antibodies in pigs of different feeding groups

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Dramatic reductions of in feed medication via immunization against enteric pathogens

PI 04

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Summary: The use of in-feed antimicrobials is coming under increased pressure in food animal production. Five field studies examined the impact of vaccines to stimulate protective immunity against pathogens commonly controlled with in-feed antimicrobials (*Lawsonia intracellularis*, a common enteric pathogen causing ileitis). Grow-finish pigs were immunized and various levels of in-feed antimicrobials used to control or prevent *Lawsonia* were removed. Performance was compared between vaccinated and matched, continuously medicated barns. Performance was improved in vaccinated/reduced medication pigs while allowing for a 50% or greater reduction in in-feed antimicrobials targeting ileitis. More than six grams of tylosin and 20 grams of tetracycline per pig were removed from finishing feeds. Up to 50% of the time period in vaccinated finishing pigs occurred without any medications in feed. Large amounts of in-feed antimicrobials were successfully removed while improving growing and finishing pig performance.

Keywords: vaccination, antimicrobials, *Lawsonia*, *Salmonella*, grow-finish

Introduction: Use of in feed antimicrobials in pork production is a controversial activity. Historically, in feed antimicrobials have been used to treat and prevent disease, as well as promote growth in normal, healthy pigs. A large percentage of pigs have typically received some type of medication for either disease treatment or growth promotion at some point in the production phase (Dewey et al., 1999). However, the continuous feeding of in-feed antimicrobials may contribute to the development of resistance in swine pathogens, a potential risk for animal and human health (Mathew et al., 2002). Active immunization against enteric pathogens may offer producers the chance to reduce in feed antibacterials while maintaining or improving production.

Materials and Methods: A common enteric pathogen of swine, *Lawsonia intracellularis*, the causative agent of ileitis, recently has had a first of its kind oral vaccine approved. Common antibacterials such as tylosin, tetracyclines and lincosin are frequently used in in-feed, water and injectable formats to treat and prevent disease associated with *Lawsonia*. Five large scale field experiments using immunization to prevent disease were performed to evaluate both the impact of vaccination on biologic performance and assess the ability to reduce the reliance of producers on in-feed antimicrobials to control a common enteric disease.

Barns of pigs were vaccinated late in the nursery phase or at placement to the finishing phase with Enterisol® Ileitis (Boehringer Ingelheim Vetmedica, GmbH), or remained as non-vaccinated, continuously medicated control groups. Enterisol Ileitis is first and only vaccine to protect pigs against ileitis which is administered orally. A total of 55 barns and approximately 46,900 pigs were immunized. There were a similar number of non-vaccinated control barns. A complete description of the study format has been described elsewhere (Kolb,2003).

Results: Growth rate (gm/day), feed efficiency (kg gain/kg feed) and mortality were significantly improved ($p < 0.05$) for groups of pigs vaccinated with Enterisol Ileitis and using reduced amounts of in-feed medications, as compared to barns receiving conventional regimens of continuous medications throughout the finishing phase. An average of more than 6 grams of tylosin were removed per pig receiving vaccination. Additionally, more than 25 grams of tetracycline were removed, per pig, in two of the five trials utilizing this antimicrobial. Performance was improved while simultaneously reducing the amount of in-feed antimicrobials.

Discussion: In this study, immunization against *Lawsonia* improved performance as compared to matched non vaccinated, continuously medicated barns of pigs. Additionally, there were large periods of time where medication free feeds were used during the finishing phase, predominantly in later finishing where the majority of feed, and thus feed medications, are consumed. This may allow for more strategically placed therapeutic medications, whether directed at enteric or respiratory disease (Walter et al., 2000). This reduction of medication use with improved performance is in contrast to those efforts where medications have been removed without immunization to address enteric pathogens remaining in the environment (Nielsen, 2002).

Immunization against *Lawsonia* may allow pork producers and veterinarians to significantly reduce the use of in-feed antimicrobials in disease preventive and growth promoting roles. This would thus reduce overall reliance on antimicrobials in pork production. The potential impact on human health of such a reduction has yet to be ascertained. However, improving production and reducing the amount of in-feed antimicrobials would provide an economic incentive for producers to reduce use of medications in feeds and improve welfare for livestock.

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PI 05 Semi-quantitative Risk Evaluation for the Occurrence of *Salmonella* spec. in Swine Herds and Slaughter Plants

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Keywords: Salmonella load, critical points, bench marking, intervention strategies

Summary: The implementation of a "Salmonella Monitoring and Reduction Programme" in the framework of the emerging national quality assurance programme (it is called the "QS-System") for food products (starting with pork) in Germany has led to the necessity to provide farmers and slaughter

plants with guidelines for a) how to identify the critical points on their own farm and in their own slaughter plant that could be “responsible” for a high *Salmonella* load of the final product (= live slaughter pigs in case of the farm or carcasses and cut meat in case of the slaughter plant), and b) how to develop a “HACCP-like” plan for a measurable reduction of the salmonella load. The paper describes the development of check lists that can be used for both benchmarking and identifying “weak points” as basis for targeted intervention strategies in the framework of continuous improvement programmes.

Introduction: Since the 1st Int. Symp. on the Epidemiology and Control of Salmonella in Pork in Ames in 1996, many studies on the prevalence of *Salmonella* antibodies in sera and meat juice (e.g. Nielsen et al., 2001; Koefer et al., 2001; von Altröck, 2001), and on the prevalence of *Salmonella* spec. in slaughter pigs (e.g. Davies, 2001) and in pigs on the farm (e.g. Gibson et al., 2001; Erdman et al., 2001; Szaszak, et al., 2002) have been carried out. There are also several reports on salmonella-specific risk factors at farm level (e.g. Kranker and Dahl, 2001; Bahnson et al., 2001; Funk, 2001) during transport, lairage and slaughter (McKean et al., 2001; Hurd et al., 2001; Limpitakis et al., 2001) as well as several studies on the risk management using the HACCP concept (Bokma-Bakker and Mul, 2001). These many research projects and published studies from many countries (the cited literature are only selected examples) as well as those on the various intervention strategies have resulted in a quite good, although not yet complete, picture of the general prevalence of *Salmonella* spec. throughout the pork chain, on the risk factors and on the possibilities to reduce the occurrence of *Salmonella* spec. in pork. This growing general knowledge on *Salmonella* prevalence estimation has ploughed the field for the establishment of regional or national Salmonella monitoring and reduction programmes in several countries.

However, one experience after the start of the national programme in Germany (Anonymous, 2003; Blaha, 2003) is that pork producers with herds that have been classified as *Salmonella* high risk herds are not satisfied with the explanation of the general knowledge on risk factors. Apart from asking for quick “silver bullet” solutions, they want to know the reasons for their high Salmonella prevalence – especially in cases, where the general hygiene and biosecurity on the farm are high and well-observed. In other words, they do not want to know what ONE can do, but what THEY can do to identify their specific *Salmonella* infection sources and risk factors for their own farm. This expectation led to the need for developing a method for an at least semi-quantitative measurement of the specific risk factors of individual farms.

Material and Methods: Two approaches to identify infection sources, contamination-infection cycles and risk factors for the high Salmonella prevalence of swine herds were combined:

1) Traditional samples of various materials on high risk farms (= “Category III farms” with a high percentage of salmonella-antibody positive meat juices) with a remarkably high hygiene status and a reliable biosecurity system, such as: feces samples from individual animals and or from pens, swabs from various materials that pigs have directly contact with (walls, troughs, pen separation material, tools, boots etc.), swabs from areas that pigs can have only indirectly contact with, as well as specimens from feed and water, were investigated culturally for *Salmonella* spec.

2) Based on the general knowledge on how to establish a HACCP programme, and on common risk factors for the occurrence of *Salmonella* spec., the production system and the production procedure of swine farms was divided into its components from animal supply, feed supply, feeding procedure, water supply, watering system, barn equipment, daily working procedures, tool usage, waste management, animal flow, shipping procedure, cleaning and disinfection etc. etc. to be able to establish per component salmonella-specific CCP's.

The culturing of the various animal and farm samples was to a) verify the theoretical assumptions of the reasons for the identified *Salmonella* sources and risk factors, and b) identify “new” sources that are not taken into account by the traditional hygiene and biosecurity programmes (hence the decision to deal mainly with salmonella-antibody positive herds that have a high hygiene status and an at least good biosecurity). Then the theoretical and “new” salmonella-specific CCP's per component

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were established and listed as the basis for a check list that provides per component and per CCP a semi-quantitative ranging of the CCP in question from ten points (best scenario) down to zero points (worst scenario).

After having started with this development at several farms, it became obvious that the same approach is reasonable for slaughter plants (developing a salmonella-specific HACCP-like system for establishing salmonella-specific CCP's per production component of the slaughter and processing procedure.

Results: The combination of both approaches has led to three semi-quantitative check lists that are currently used for their evaluation and improvement by applying them to herds with high and low *Salmonella* loads (as determined by the German national Salmonella programme). One check list is used just for finisher herds (in disrespect whether it is in a closed system or not), one check list is used for sow herds that supply finisher herds and or breeding herds with production or replacement animals, and one check list for slaughter plants starting with the transport and lairage procedure.

Conclusions: Although the check lists are still in the process of being tested and evaluated, two preliminary conclusions can already be drawn as far as the identification of weak points is concerned:

- 1) There are many salmonella-specific "hygiene breaches" even in the cleanest farm environments that have been overlooked so far since they are not tackled with by the traditional cleaning and disinfection procedures (dust in fan in- and outlets, dirt under pen separation walls or under feed and/or water troughs, never washed scales used for all pigs once or twice during a production cycle, etc. etc.). This means that improving the quality of the general hygiene and biosecurity is important, but additionally to these measures, a targeted (check list aided) search for "non-traditional" salmonella-specific weak points is inevitable.
- 2) Identifying commonly known risk factors (= low hygiene farms) or "new" salmonella-specific risk factors (high hygiene with "hidden" hygiene breaches) does not necessarily mean that the Salmonella load is "automatically" high, as many may expect. However, this does not mean that the developed check lists are of no value for salmonella programmes. Their value is that a) farm and slaughter plant managers that have an acute "Salmonella problem" can use them to detect "weak points" as basis for improvement measures, and b) farm and slaughter plant managers that have no acute problem, but a higher risk of having an acute problem can use the check list results for proactively reduce the identified risk.

The three check lists have been used so far on many and several slaughter plants. It has turned out that farm owners and managers as well as slaughter plant managers are highly interested in both the identification of salmonella-specific weak points and in the benchmarking that is provided by the semi-quantitative approach of the check lists, the latter is appreciated in particular by managers of production systems with various farms belonging to one pork production system and by the managers of the slaughter and processing industry when several plants belong to one company.

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***Salmonella* Typhimurium CARRIAGE at slaughter AFTER an enterocolitis outbreak in a swine herd**

PS 01

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Summary: The aim of this study was to estimate the prevalence of slaughter pigs carrying *Salmonella* Typhimurium after a enterocolitis outbreak in a commercial pig farm. A cross-sectional study was done during the slaughter of a batch of 86 animals. Mesenteric lymph nodes from 43 pigs were collected and pre-enriched in buffered peptone water (1:10) overnight at 37AC Afterwards, aliquots of 1mL and 0.1mL were transferred to selenite-cystine and Rappaport-Vassiliadis broth, respectively. A loopful of each sample was streaked onto XLT4 and brilliant green agar plates, which were incubated at 37AC for 24 hours. *Salmonella* was cultured in 23 out of 43 collected samples (53.48%). *Salmonella* Typhimurim (13 strains) and *Salmonella enterica* subs. *enterica* 1,4,5,12:i:- (10 strains) were isolated. These results indicated that the slaughter of pigs from batches previously affected by enteric salmonellosis may represent a high risk for pork contamination, since there is an positive association between infected pigs before slaughter and carcass contamination.

Keywords: Carcass, Lymph nodes, Outbreak, Pigs, Pork

Introduction: In the last decades, the pattern of *Salmonella* serotypes involved in clinical salmonellosis in pigs has changed in some countries. There has been a continuous increase in the incidence of salmonellosis caused by non-adapted serotypes, such as *Salmonella* Typhimurium, compared to those caused by *Salmonella* Choleraesuis. Some reports have already shown the long-term shedding of *Salmonella* Typhimurium after experimental inoculation (Wood and Rose, 1992; Van Winsen et al., 2001.). The aim of this study was to estimate the prevalence of pigs carrying *Salmonella* at slaughter, which were originated from a herd previously affected by an enterocolitis outbreak confirmed as salmonellosis.

Material and Methods: The prevalence estimation was determined by a cross-sectional study in animals originating from a herd that had shown clinical signs of enterocolitis. Salmonellosis was diagnosed by suggestive clinical signs (such as diarrhea in the growing and finishing phases, high morbidity/low mortality, weight loss-15kg lighter at slaughter), pathological findings and isolation of *Salmonella* Typhimurium from feces and organs of affected animals. Clinical signs were controlled when gentamicin was included in the feed from 70 to 77 days and from 110 to 117 days. Sample size was estimated assuming an expected prevalence of 50%, which permitted an estimation of prevalence within 10% accuracy at the 95% confidence level. Mesenteric lymph nodes from 43 pigs were collected and pre-enriched in buffered peptone water (1:10) overnight at 37AC. Afterwards, aliquots of 1mL and 0.1mL were transferred to selenite-cystine broth and Rappaport-Vassiliadis broth, respectively. A loopfull of each sample was streaked onto XLT4 and brilliant green agar plates, which were incubated at 37AC for 24 hours. Suspected salmonella colonies were transferred to tripe-sugar-iron agar and lysine-iron agar slants. All strains presumptively identified as *S. enterica* were confirmed by slide agglutination test with poli-O and poli-H antiserum. All *Salmonella* isolates were serotyped as described by Popoff and Le Minor (1997).

Results: *Salmonella enterica* was cultured in 23 out of 43 collected samples (53.48%; 95% CI: 42.94:64.02%). *Salmonella enterica* serovar Typhimurim (13 strains) and *Salmonella enterica* subs. *enterica* 1,4,5,12:i:- (10 strains) were identified. Although the phase reversal was not achieved in these 10 strains, the antigenic formula analysis indicate they are also *Salmonella* Typhimurium.

Discussion: Our results indicated that *Salmonella* Typhimurium can persist in mesenteric lymph nodes of slaughter-age pigs naturally infected in the farm during growing and finishing phases. These results are in accordance to previous data demonstrating long-term carriage of *Salmonella* Typhimurium after exposure (Wood and Rose, 1992). Considering that stress (particularly associated with transportation) may increase *Salmonella* shedding by infected animals, which might contribute to the spreading of the agent, we were unable to estimate the prevalence of infected animals on farm. Indeed, recent reports have demonstrated that slaughter-age pigs may become infected in a short period after placed in a contaminated environment (Hurd et al., 2001). As the cross-contamination of pork during slaughter is strongly linked to the prevalence of animals carrying *Salmonella*, these results showed that the slaughter of pigs from batches previously affected by salmonellosis may represent a risk-point for pork contamination.

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Pork safety and quality through livestock welfare: 2. Pig welfare during pre-slaughter and stunning

PS 02

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Summary: Pig welfare from farm to slaughter can be defined by the stress the animals undergo when faced with new environments. Recent reports concerning animal welfare during transport and at the time of slaughter in Greece indicate that corrective actions should be taken by the Greek authorities in particular to supervision and monitoring the situation of transportation, resting and stunning of animals. Slaughter pigs are currently subjected to number of handling practices from the moment they leave their fattening pen until they stunned at slaughter. New management practices taking into account animal welfare during pre-slaughter and stunning (fasting, loading, transport, lairage, stunning system) must be adopted in order to limit animal stress from farm to slaughter. Some of these practices (equipment, logistics, training of the personnel) to be implemented are discussed. Apart of the issue of the animal welfare, the lack of adequate preparation of pigs on farm (fasting) and the use of poor handling systems throughout the pre-slaughter period also lead to carcass depreciation and meat quality defects (PSE, DFD) which reflect in great economic losses for the industry.

Keywords: pigs; antemortem handling; critical points; PSE; DFD

Introduction: Major deficiencies in the field of animal welfare during transport and at the time of slaughter in Greece were recorded by the FVO/EC in 1998 and 2000 (Report 1998, 2000). New measures taking into account animal welfare during pre-slaughter and stunning (fasting, loading, transport, lairage, stunning system) must be adopted in order to limit animal stress from farm to slaughter (Ramantanis, 2002).

Critical points within the pre-slaughter period:

I. Farm: Preparation for transport/ Loading: Currently, a feed withdrawal of 16-24 h before slaughter is recommended in practice (Ramantanis, 2002). Fasting for at least 12 h before loading decreases the risk of mortality during transport (Chevillon, 2000). Long fasting periods, when associated to long transports or lairages, would tend to decrease the incidence of PSE meat and increase the prevalence of DFD meat (Eikelenboom et al., 1991). Loading pigs onto the truck is considered the most critical stage of the transport stage. The use of a lift makes the pigs easier to handle and prevent the handlers from using coercion on them. If a ramp is necessary it should have an angle of $< 20^{\circ}$ (Christensen et al., 1994).

II. Transport: Vehicle design/ Stocking density/ Transport time and distance/ Unloading: To optimize the transport conditions, the transport vehicle should have a covered and insulated deck (ceiling and sides), effective mechanical ventilation and ventilation openings both low down and high up semi-automatically adjusted from the driver's cabin on the sides, as well as hydraulic upper deck, mobile compartment dividers and a non-skid rubber surface on the floor and a sprinkling equipment (Christensen and Barton-Gade, 1996). The current legislation in Europe (95/29/EC) specify that loading density for pigs of around 100 kg should not exceed 235 kg/m² (0.425 m²/100kg) and that a maximum increase of 20% (0.510 m²/100kg or 196 kg/m²) may also be required depending on the meteorological conditions and journey time. In most EU countries stocking densities range from 0.35 to 0.39 m²/100 kg and go up to 0.43-0.50 m²/100 kg in Germany. Market pigs can experience higher mortality (heat stress), injuries (bruises) and lower meat quality if the space allowed is not appropriate. In a EU survey, the majority of pigs within all countries travelled less than about 3 to 2 h with average distances of 100 km or less. (Barton-Gade,

2001). Transports lower than 2h increased the severe PSE meat incidence by 2.3% in Spain, whereas, pigs transported >2h may be more likely to develop DFD meat (Gispert et al., 2000). The use of the hydraulic lift to unload pigs increases the easiness of handling and shortens the off-load time.

III. Slaughterhouse: Lairage/ Moving pigs to stunning/ Stunning methods and their impact on welfare:

Under normal conditions of ambient temperature and humidity a resting time of 2-3 hours in lairage pens is usually regarded as a fair compromise between animal welfare, skin blemish score, meat quality and abattoir economics (Warriss et al., 1998). The movement of pigs up to the stunning point is very stressful because they are handled fast and in small groups. To decrease this stress a new low stress system (LSS) has been developed, which incorporates smaller pens holding only 15 pigs and automatic push gates to move the animals. Before the stunner the groups are divided into three groups of 5 pigs each for CO₂-stunning (Barton-Gade et al., 1992). Whatever electronarcosis or cardiac-arrest system are used, the animal welfare must always be assured (Chevillon, 2000). The LSS system promotes the animal welfare whereas the CO₂-stunning reduces skin damage, blood splash and PSE incidence in comparison to electric stunning (Channon et al., 2002).

Conclusions: New management practices taking into account animal welfare issues from farm to slaughter must be adopted at each production segment (farmer, transporter, packer). In the antemortem period, good welfare usually results from careful handling of animals that reduces stress and trauma. In contrast, poor antemortem handling leads to stress and results in poorer meat quality. Appropriate training and information related to animal welfare should be provided for all relevant veterinary personnel so that the official veterinary staff can check the application of Animal Welfare legislation professionally.

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Salmonella enterica in pork: Prevalence in the environment, carcasses and by-products in the slaughterhouse of a vertically integrated company (2001-2002)

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Summary: The study determined the prevalence of *Salmonella* spp. in the slaughterhouse environment, and products of a vertically integrated company in Crete. From 30/11/2001 to 11/6/2002 we visited the plant 7 times and collected 250 samples. The pigs originated from the company's near by farm. During operations and after 75-100 pigs were processed we collected 185 samples. The prevalence of *Salmonella* spp in 4 visits was 9.09, 15.22, 6.38 and 2.13 % respectively. The prevalence in scalding tank overflow water, floors, workers' hands, workers' knives, livers tank, pork carcasses, livers, and tongue surfaces and ileocecal lymph nodes and caecal contents samples was 14.2% (range: 0-50), 11.1% (0%-50%), 0%, 0%, 0%, 2.5% (0-10), 2.5% (0-20), 2.5% (0-20), 15% (0-40) and 35% (0-80) respectively. The prevalence in 3 visits and collection of 66 samples before the onset of operations was 22.7, 31.8 and 9.1 % respectively. The prevalence in the lairage area, the drains, cutting saw, knives, plastic door panels, workers aprons and offal baskets surfaces was 44.4% (range 0-67), 33.3% (20-40), 0%, 11.1% (0-33.3), 8.3% (0-33.3), 22.2% (0-33.3), and 11.1% (0-33.3) respectively. Only *S. Infantis* has been isolated. Caecal prevalence of 60-80% did not correlate with increased prevalence on carcasses and livers in the same sampling day. Isolated strains were resistant to Rifampin and Nitrofurantoin and sensitive to 35 other antimicrobials. Prudent use of antimicrobials mostly for therapeutic purposes was initiated in 1999. Rifampin and Nitrofurantoin have never been used.

Keywords: Contamination, prevalence, pork, Greece, antimicrobials

Introduction: Salmonellosis remains a global human health problem including Greece and the island of Crete (Schmidt and Tirado 2001; Maraki et al., 2003). Foods of animal origin are most commonly implicated as a source with pork contributing to 5-30% of all foodborne cases. The incidence rate/100,000 inhabitants in Greece was 18.4, in 1994, and 8.8 in 1998. *S. Enteritidis* frequency was 79.2% in 1993 and 68.7% in 1998. *S. Typhimurium* was 14.1 and 24.9% and *S. Infantis* 3 and 0.5% respectively. In Crete in 2001 and 2002 the frequencies for *S. Enteritidis* were 63.8 and 73.8 %, for *S. Typhimurium* 17 and 8.7%, and for *S. Infantis* (3rd most common) 2.1 and 2.9% respectively. *S. Infantis* was not isolated in 1995-8 (Schmidt and Tirado 2001; Tselentis, 2003). In this study we investigated the prevalence of *Salmonella* spp., in the slaughterhouse of a vertically integrated company in Crete, processing >80,000 pigs/year.

Materials and Methods: Slaughtered pigs were raised in a near by farm and were transported to the slaughterhouse's stable 24 hours before processing. Using swabs we collected surface samples from the environment and products before and after the processing started. The methodology for isolation/identification of *Salmonella* spp., has been reported (Limpitakis et al., 1999a).

Results: During operations 184 samples were collected. The prevalence of *Salmonella* spp for each of 4 visits (11/2001 and 1,4,5/2002) was 9.1, 15.2, 6.4 and 2.1 % respectively. The prevalence in samples from floors, workers' hands, workers' knives, scalding tank overflow water, liver tank water, pork carcasses, livers, and tongue/larynx surfaces, caecal contents and lymph nodes were 11.1(0-50), 0, 0, 14.2 (0-50), 0, 2.5 (0-10), 5 (0-20), 5 (0-20), 35 (0-80), and 15 (0-40) % respectively. *Salmonella* spp was found in 21.2% of 66 environmental samples collected in 3 visits (3,4,6/2002) before the onset of operations. The prevalence for each visit was 22.7, 31.8 and 9.1 % respectively. The prevalence in the lairage area, drains, cutting saw, knives, plastic door panels, workers aprons and offal baskets surfaces

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was 44.4 (0-67), 33.3 (20-40), 0, 11.1 (0-33.3), 8.3 (0-33.3), 22.2 (0-33.3), and 11.1 (0-33.3) % respectively. Only *S. Infantis* has been isolated which was resistant to Rifampin and Nitrofurantoin but not to 35 other antimicrobials.

Discussion: Limpitakis et al., (1999) study of 2 slaughterhouses in N.Greece reported higher prevalence of *Salmonella* spp during the warmer then cooler months. This was attributed to greater stress of animals during transportation and larvae in the hot Greek summers and probably to loading more pigs per truck than usually, to meet the increased tourist season demand for meat. Environmental *Salmonella* spp., multiplication was not excluded. In multivariate logistic regression models with random effects the proportion of *Salmonella* positive carcasses in a sampling round was associated among others with the slaughterhouse, the period of the year the sampling was done, the sampling round (early or later in the slaughtering time), the sampling day and the results from testing the various environmental samples. (Limpitakis et al., 2001c). Our study was too short to allow identification of weather effects. Climatic conditions in Crete are milder and less fluctuating than in N. Greece to show impact on the ecology of *Salmonella* spp. Berens et al., (1997) estimated that about 70% of carcass-contamination results from pigs themselves being carriers and about 30% from other carrier pigs. In two visits 60 and 80% of the caecal contents and 40 and 20% of lymph nodes were positive. These figures did not correlate with environmental and product *S. Infantis* prevalence those particular days. In 4 visits the caecal prevalence was 60, 80, 0 and 0% respectively, indicating the need for a better understanding of *Salmonella* spp., ecology in the farm. The isolation of only *S. Infantis* differs from 22 serotypes identified by Limpitakis et al., (1999), with *S. Infantis* (6.7%) being the 5th most frequent. No *S. Enteritidis* was isolated. *S. Typhimurium* DT 104 was isolated for the first time in the country. The sensitivity of *S. Infantis* to 35 antimicrobials is of interest. Since 1999 the company limits the use of antimicrobials. Tetracyclines, sulfonamides and microlides are not used. Those used are rotated annually and their use is interrupted 4 months before slaughtering. Rifampin and Nitrofurantoin have never been used. Limpitakis et al., (1999) observed resistance to many important antimicrobials. Resistance of *Salmonella* spp, from diarrhoeal cases in Crete, to Tetracycline, Ampicillin, Chloramphenicol, Sulfamethoxazole/Trimethoprim, Gentamycin and Neomycin was exhibited by 40.7, 36, 4.7, 3.5, 0.4, and 0.2% of the strains respectively.

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Microbiological and Chemical Quality of Minced Meat Packaged in Modified Atmosphere at +1-2°C.

PS 04

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Summary: The aim of the work was to determine the shelf-life and the microbiological quality of minced meat packaged in modified atmosphere (MAP, 20% O₂, 5% CO₂, 75% N₂). Changes in the microflora and in the chemical characteristics were monitored for a total period of 10 days. Different samples of minced pork meat and of minced mixed pork and beef meat (50% w/w) were packaged and stored at +1-2°C. The results demonstrated that the minced meat samples studied had a 6 days shelf-life; the microbiological quality, the pH value and the total volatile nitrogen (TVN) were acceptable. After the sixth day, both minced meats started to brown, becoming unacceptable by the consumers.

Keywords: spoilage, browning, safety, shelf-life, storage.

Introduction: Minced meat is a good substrate for the growth of different micro-organisms. Despite the packaging in Modified Atmosphere (MAP) and the storage at 4°C, the psychrotrophic bacteria can grow and produce browning and off-flavours. The effects of microbial contamination are the reduction of shelf-life and the potential compromise of the safety of the product. *Salmonella* spp., *Campylobacter* spp., *Escherichia coli*, *Yersinia enterocolitica* are often isolated from minced meat. They originate from personnel, equipment and slaughtering utensils and surface of the meat (Northjé et al., 1989). For these reasons, many studies have focused on defining the hygienic quality of minced meat (Northjé et al., 1989; Mutti et al., 2001; Marino et al., 1995). The aim of this work was to determine the microbiological quality and the shelf-life of minced meat investigating the behaviour of the microflora and the chemical changes occurring during storage under modified atmosphere and refrigeration at 1-2°C.

Materials and methods: Two different lots of minced meat were investigated; minced pork (P) meat and minced mixed pork (50% w/w) and beef (50% w/w) (PB) meat were packaged and stored at +1-2°C for ten days. The samples were packed in trays OPET, with 66 ml O₂, 25 ml CO₂ and 9 ml N₂ /litre atmosphere. The covering films for MAP were PEEDOH-PE. Samples were examined at 0, 3, 5, 10 days to determine the increase of the microorganisms. Total aerobic counts were determined in Plate Count Agar (Oxoid, Italy) after incubation at 20°C for 2 days; Lactic Acid Bacteria (LAB) were determined in de Man Rogosa Sharpe (MRS, Oxoid, Italy) after incubation at 30°C for 5 days under microaerophilic conditions; *E. coli* and coliforms were detected in Coli ID (bioMerieux, France) after incubation of two days at 37°C. The presence of *Listeria monocytogenes* was evaluated by the ISO/DIS method (1990) and the presence of *Salmonella* spp. by the ISO/DIS method (1991). The chemical changes were evaluated determining the pH, using a digital ph-meter (Beckman, mod. 3560, Glenrothes, UK), the total volatile nitrogen (TVN) according to Pearson (1973), the number of peroxides (PV) according to Shanta et al., (1994) and the malondialdehyde (MDA) concentration according to Chiesa et al., (1999, 2000). The L*a*b* Hunter parameters were also evaluated by using a tristimulus colorimeter (Chromameter-2 Reflectance Minolta, Osaka, Japan).

Results: In both types of sample, the total aerobic flora increased until the end of the experiment. The growth was favoured by the presence of oxygen inside the package and the maximum values approached 10⁸ CFU/g. LAB increased in both minced meats until the 7th day and reached values

of 1.4×10^8 CFU/g in the P and 6.0×10^8 CFU/g in the PB meat. The greatest increase was observed between 5 and 7 days of storage. At 10 days LAB decreased in both meats. Coliforms did not reach values higher than 4.3×10^3 CFU/g. No *L. monocytogenes* or *Salmonella* spp. were detected in the samples tested. The pH values after 7 days reached 5.76 in P and 5.55 in BP meat. The changes were due to the combined effect of the increase of the bacterial concentrations and of the production of volatile nitrogen compounds. The values of TVN in both the P and PB samples varied from 21.7 to 24 mgN/100g. The level of TVN was higher than the ones obtained by Pearson (1973), that determined that fresh beef meat could contain maximum values of 17 mgN/100 g. The peroxides values reached maximum levels at 7 days for PB, and at 3 days for P samples. After this time they decreased. The value of peroxides observed (less or equal to 5.7 meq O_2 /kg fat) can be considered acceptable according to the data of Church & Wood (1992). Viceversa Chizzolini et al. (1998) suggested 2-4 meq O_2 /kg fat as acceptable level of peroxides values. MDA was measured after 7 days of storage for both P and PB samples. The values were similar to the ones obtained by O'Grady et al. (2000) in minced beef meat packaged in different MAP. Similar results were also obtained by Smiddy et al. (2002) and Formanek et al (1998). The minced meat P and PB demonstrated a good colour retention only in the first 5 and 7 days of storage, while browning appeared within 7-8 days of storage. The PB meat maintained a brilliant red colour longer than the P meat.

Conclusions: Data demonstrated that both minced pork meat and minced beef/pork meat were safe. The spoilage started within 3 days of storage but could be measured at 7 days. This period should be considered the shelf-life of both products, because sensory and chemical analysis demonstrated a change in colour, an increase of TVN, number of peroxides and MDA concentration after that time. The spoilage was more predominant in mixed beef/pork meat.

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Mechanisms of resistance in *Salmonella enterica*.

PR 01

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Summary: The aim of this study was to promote resistance in *Salmonella enterica* serovar Enteritidis, Typhimurium, and Virchow to commonly used antibacterials and to identify mechanisms underlying any resistance obtained. Strains were exposed to various biocides and following each passage adaptive resistance was recorded. Permeability changes in the outer membrane, including LPS, cell surface charge and hydrophobicity and efflux were investigated as possible resistance mechanism candidates. The outer membrane and LPS bands were analysed by SDS-PAGE and visualised by Coomassie blue and silver staining. The cell surface charge and hydrophobicity were investigated employing microelectrophoresis and microbial adhesion to hydrocarbons assay, respectively. Efflux activity was examined by comparing resistance in pre- and post-adapted strains in the presence of reserpine. The outer membrane and LPS did not reveal any significant changes. Most of the parent strains were not significantly hydrophobic, whereas adapted were. An active efflux system was associated with benzalkonium chloride and chlorhexidine resistance.

Keywords: Adaptive resistance, *Salm.* Enteritidis, *Salm.* Virchow, Antibiotics, Biocides.

Introduction: Bacteria continue their natural evolution and can develop resistance mechanisms to antibacterial drugs at an incredible speed. This has created a growing public health problem, which may be exacerbated by the commonplace and often unnecessary addition of biocides into household products. Antimicrobial resistance among human and veterinary bacterial pathogens develops through one or more several possible mechanisms including permeability changes in the cell membrane, active efflux, enzymatic inactivation or destruction of the antimicrobial, alteration of the target site of antimicrobial action and creation of altered enzymatic pathways among others. The majority of antimicrobials used in the veterinary medicine can be blocked by one of these or a combination of these mechanisms (White, 2000). Thus, this study was aimed to understand how bacteria manage to endure antimicrobial action by investigating the mechanisms underlying resistance.

Materials and Methods: Antimicrobial susceptibility testing, the reserpine effect and bacterial adaptation: Standard broth dilution method was carried out using a two-fold dilution of each antibacterial agent (Loughlin et al., 2002) and reserpine for the MIC determination of approximately 10^8 bacteria. The MICs of pre- and post- adapted *Salmonella enterica* cultures were also obtained in the presence and absence of reserpine 20µg/ml together with the antibacterials. After the overnight incubation period at 37 °C bacterial growth was assessed by observing turbidity in the media. Bacterial adaptation was performed according to the technique described by Joynton et al., 2002. Following adaptation, random amplification of polymorphic DNA (RAPD) was employed to confirm the molecular identity of the strains. (Hopkins and Hilton, 2001).

Preparation and analysis of outer membrane proteins (OMP) and lipopolysaccharide (LPS): Outer membrane extracts were prepared by a method based on Lambert et al., 1982. Each membrane suspension (30µl) in loading buffer was electrophoresed at 200 V for 42 minutes in an 11% polyacrylamide gel using Bio-Rad Mini Protean II apparatus. The LPS bands were visualised either by silver staining (Fomsgaard et al., 1990) or by Coomassie blue stain.

Cell Surface Hydrophobicity (CSH) & Charge: The cell surface hydrophobicity of *Salmonella enterica* strains was determined by microbial adhesion to hydrocarbon (MATH) assay employing n-hexadecane

as the hydrocarbon phase (Loughlin et al., 2002). The cell surface charge was determined by particle microelectrophoresis using a Zetamaster Particle Electrophoresis Analyser, (Brookhaven Instruments) to measure the zeta potential. Bacterial cells were grown in nutrient broth to stationary phase and suspended in 1mM (KCL) at a concentration of approximately 1×10^7 cells/ml.

Statistical Analysis: The data for MATH and microelectrophoresis were checked for normality using the Kolmogorov-Smirnov test. The MATH and microelectrophoresis results were statistically analysed using a single factor analysis of variance (ANOVA) and Fisher LSD post hoc analysis. All analysis was carried out using the Statistica Program.

Results: Antimicrobial Susceptibility Testing, the Reserpine Effect and bacterial adaptation:

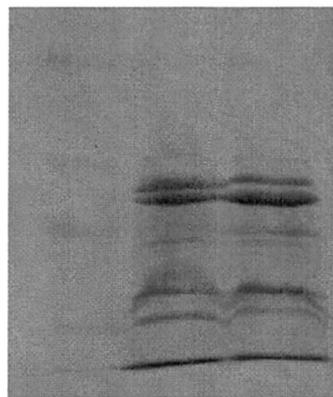
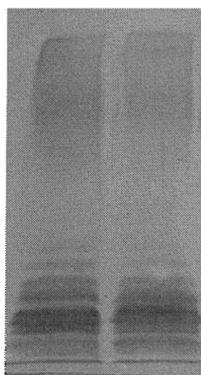
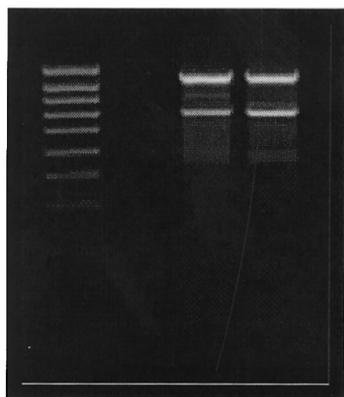
Resistance was obtained in all strains investigated within 6 days of gradual exposure and strain continuity was confirmed by RAPD profiling (Figure 1). Results strongly indicated the operation of an efflux pump system in benzalkonium chloride (BKC) and chlorohexidine (CHX) resistance (data not shown).

Preparation and analysis of outer membrane proteins and LPS: No significant difference in outer membrane and LPS profiles were apparent between pre- and post-adapted strains. Figure 2a represents TLN-adapted *Salmonella* Virchow by silver staining and figure 2b shows pre- and post-adapted *Salm.* Virchow strains visualised by Coomassie brilliant blue.

Cell surface Hydrophobicity and Charge: Benzalkonium chloride adapted-*Salm.* Enteritidis, *Salm.* Typhimurium and *Salm.* Virchow, as well as erythromycin-adapted *Salm.* Enteritidis and *Salm.* Typhimurium showed significant changes in hydrophobicity. More specifically, parent strains did not reveal any significant hydrophobicity, whilst adapted did (data not shown). All strains investigated shown to carry a negative charge. No significant changes between pre- and post- adapted strains were apparent (data not shown).

Figure 1: RAPD profile from *Salm.* Typhimurium pre- (Lane 1) and post-adapted to triclosan strains (Lane 2).

Figure 2a: Coomassie blue stained pattern between pre- (Lane 1) and post-adapted to triclosan *Salm.* Typhimurium strains (Lane 2). 2b: Silver stained pattern between pre- (Lane 1) and post-adapted to triclosan *Salm.* Virchow strains (Lane 2).



Discussion: The presence of an active efflux pump, the outer membrane and LPS bands and the cell surface hydrophobicity and charge were examined as possible candidates of resistance. Efflux pumps play a vital role in the establishment of resistance of Gram-negative bacteria to a panel of antimicrobial agents (Rosenberg, 2000). More specifically, it was found that adapted strains returned to their parent MIC in the presence of reserpine, which consequently suggests the up-regulation of an efflux protein. In this study, there was a strong indication of BKC and CHX efflux mediated resistance. Resistance to BKC mediated by efflux pumps has been previously documented by Aase et al., 2000.

POSTER PRESENTATIONS

The mechanisms of CHX resistance remain unclear. Fang et al., 2002 proposed the possibility of a cationic efflux pump in CHX-adapted *E. coli*. Lipopolysaccharides are the main components of the outer membrane of Gram-negative bacteria and are responsible chiefly of the cell impermeability characteristics. Outer membrane and LPS profiles did not reveal any significant changes in all *Salmonella enterica* strains investigated. This is rather unusual since a number of reports support that resistance in Gram-negative bacteria might be associated with changes in outer membrane including LPS (Loughlin et al., 2002). One of the perceptible effects of the biocidal interaction with the bacterial cell is a change in cell surface hydrophobicity (Maillard, 2002). In this study, *Salm.* Enteritidis, *Salm.* Typhimurium and *Salm.* Virchow adapted to BKC, as well as *Salm.* Enteritidis and *Salm.* Typhimurium adapted to ERY were significantly more hydrophobic than the parents. In addition there was no significant difference in the cell surface charge between parent and adapted strains and no correlation between hydrophobicity and charge was evident as proposed by Wilson et al., 2001.

Conclusions: In summary, increased cell surface hydrophobicity and the presence of an active efflux pump could facilitate the acquisition of antibacterial resistance in *Salmonella enterica*, providing cross-resistance to a range of antibiotics and biocides. The emergence of bacterial antimicrobial resistance might be associated with the imprudent use of antibacterials in agriculture. White (2000) proposed that pork producers and veterinarians must treat ill animals; however misuse of antimicrobials in swine could negatively influence consumer confidence in pork products.

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PR 02 Antimicrobial resistance profile and genetic diversity of *Salmonella enterica* serotypes Typhimurium and Muenchen

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Summary: The aim of this study was to compare the antimicrobial resistance pattern and genetic diversity of *Salmonella enterica* serotypes Typhimurium and Muenchen from human and swine. Previously, we reported two predominant multi-drug resistant (MDR) patterns common among *Salmonella* isolates from swine. In this study we report serovar Muenchen, to have MDR pattern similar to Typhimurium and with expanded spectrum in swine (AmCmStSuTeKm). This pattern is more frequent among isolates from swine (with 46% frequency) while most of isolates from human were pansusceptible (only one isolate with MDR to 10 antimicrobials). Genotyping using PFGE revealed swine and human isolates clustered separately from each other. We identified class-I integrons among nine *S. Muenchen* isolates from swine and single isolate from human using polymerase chain reaction (PCR). We propose that interserovar exchange of resistance genes might be responsible for emergence of MDR strains among serovars not previously showing MDR. Further molecular investigations are underway.

Keywords: Multidrug resistance, genotyping, pigs, fingerprinting, integrons

Introduction: Multidrug resistant (MDR) strains of *Salmonella* Typhimurium have been isolated from pigs (Gebreyes and Altier, 2002). Another important serotype is Muenchen, which is in humans and accounts for 1.6% of the cases in the United States (CDC, 1997). Also, foodborne disease outbreak have been traced to improperly processed pork products or primarily linked to pig farms (Murase et al., 2000). Therefore, we decided to ascertain the antimicrobial resistance profile between the *S. Typhimurium* and *Muenchen* isolates from humans and swine and compare genotypes from different sources in order to understand their genetic similarity and/or diversity.

Materials and Methods: We tested *S. Typhimurium* isolated from 365 human clinical, 56 swine clinical and 484 swine non-clinical isolates and *S. Muenchen* from 40 human and 28 swine isolates. The isolates were tested for 14 antimicrobials by Kirby-Bauer and/or Vitek calorimetric methods using the NCCLS standards for *Enterobacteriaceae* family. The antimicrobials with their abbreviation used are: ampicillin (Am), amoxicillin-clavulanic acid (Ax), amikacin (An), chloramphenicol (Cm), ceftriaxone (Cro), ciprofloxacin (Cip), cephalothin (Cf), gentamicin (Gm), kanamycin (Km), Piperacillin (Pip), streptomycin (St), sulfamethoxazole (Su), tetracycline (Te) and trimethoprim-sulfamethoxazole (Ts). Genotyping was done by Pulsed field gel electrophoresis (PFGE) and fingerprint analysis was done by using the Bionumerics software. PCR was used to detect the presence of integrons, bla_{pSE1}, bla_{TEM}, aphA1-lab, tetA and tetB genes.

Results: *S. Typhimurium* isolates from swine had a higher frequency of resistance but the human clinical isolates showed resistance to more antimicrobials. Resistance was also seen against ceftriaxone (1.6%) and ciprofloxacin (single isolate) among the human isolates.

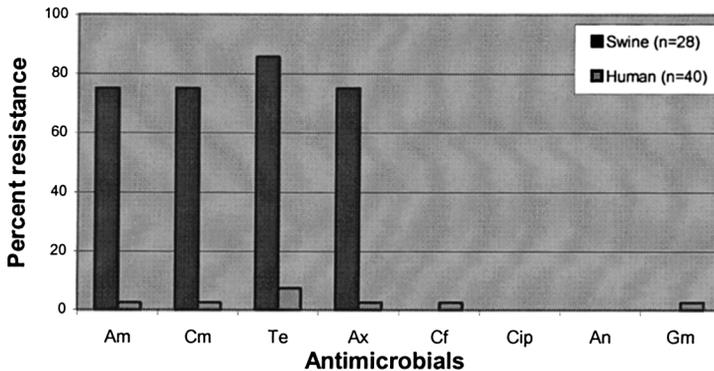


Figure 1. Antimicrobial resistance profile of *S. Muenchen* from human and swine cases.

PFGE of the three groups showed isolates from clinical cases clustered together and separate from the research isolates. Twelve isolates out of 13 *S. Muenchen* isolates tested for 12 antimicrobials from swine showed AmCmStSuTeAxKm resistance pattern. The human isolates were susceptible to most of the antibiotics (Figure: 1). Dendrogram revealed the swine isolates grouped together in a cluster and separate from the human isolates. PCR detected the presence of integron in 9 swine isolates. We also detected *aphA1-lab*, *tetB* genes from swine and *bla_{PS}E1* gene in one human isolate.

Discussion and Conclusions: Integron detected among *S. Muenchen* isolates seen in this study in swine has never been reported so far. This resistance pattern is similar to the one found above in Typhimurium though *Muenchen* had both kanamycin and chloramphenicol resistance at the same time. This indicates that both the serotypes are capable of exchanging resistance genes among each other. Detailed molecular studies are being carried out at present. Though most of the human isolates were susceptible, a single isolate was resistant to 10 out of 12 antibiotics and also had the gene for integron. Fingerprinting analysis showed separate cluster formation between the swine and human clinical isolates showing genetic diversity based on host. The most common resistance pattern and phage type in swine non-clinical isolates was not seen at all in the clinical cases. Based on fingerprinting results, we observed phenotypic and genotypic dichotomy based on the type of isolate (clinical or non-clinical) and not on the host involved. This indicates that the predominant MDR strains of Typhimurium from swine may not be important causes of foodborne illnesses in humans and that there are other foci of infections as supported by recent model.

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PR 03

Antimicrobial susceptibility of *Salmonella* isolated pig carriers

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Summary: Ninety-six *Salmonella* isolates from healthy carrier pigs were obtained from a survey in pig farms of Catalonia (Spain). Isolates were serotyped and examined for their antimicrobial susceptibility against a panel of 18 antimicrobial agents. Only isolates having different phenotypic and antimicrobial susceptibility characteristics were considered. With this restriction, we considered to have 62 different strains belonging to 17 serotypes. The most common serotype was Anatum (16.1 %) followed by Rissen (14.5 %), Typhimurium (11.3 %), Derby (9.7 %), Tilburg (8.1 %), Goldcoast (8.1 %) and Typhimurium variant 4,5,12:i:- (6.5 %). Others 10 serotypes were also isolated less than three times each. Antimicrobial susceptibility analysis showed that the highest level of resistance was against tetracycline (68.8 %). Sixty-two percent of the strains showed resistance to three or more antimicrobial agents and 46% were resistant to five or more drugs. The maximum number of compounds to which two strains were resistant was 10 (corresponding to a 4,5,12:i:- strain). None of the strains was resistant to colistin or ceftriaxone and 12 strains were susceptible to all antimicrobial agents tested

Keywords: S. Typhimurium, Multiresistance, Swine, colistin. Susceptibility

Introduction: Development of antimicrobial resistance is one of the most important health issues for public health authorities worldwide. It has been much debated about the possible role of animals as reservoirs of these resistances, especially for enteric pathogens such as *Salmonella*. In previous reports we showed that a high proportion of *Salmonella* isolates from pig faeces in Catalonia were multi-drug resistant (Mateu *et al.*, 2002). However, those studies were related to clinical cases and were not designed as a population survey. In the present report, we present the results of a survey conducted in finishing and sow units to determine the frequency of different *Salmonella* serotypes and the antimicrobial susceptibility profiles of the isolated strains.

Material and methods: One hundred and thirteen finishing units and 74 sow units of Catalonia (Spain) were bacteriologically examined to determine the prevalence of *Salmonella* serotypes. In each herd, twenty faecal samples were collected individually (sows) and twenty pooled fecal samples (5x5 gr faeces) were taken in finishing pens. All samples were inoculated in Rappaport-Vassiliadis broth and incubated at 42 °C performing subcultures at 37 °C onto XLT-4 agar at 24 h and 48 h. Suspect colonies were identified by means of biochemical tests and all strains classified as belonging to the *Salmonella* genus were sent to the National Reference Center for Salmonellosis (Algete, Spain) for serotyping. In parallel, isolates were examined for their antimicrobial susceptibility against a pannel of 18 antimicrobial agents (ampicillin, amoxycillin-clavulanic acid, ceftiofur, ceftriaxone, streptomycin, gentamycin, neomycin, apramycin, tetracycline, sulphonamides, sulphonamides+trimethoprim, cloramphenicol, nalidixic acid, flumequine, enrofloxacin, ciprofloxacin, colistin and nitrofurantoin) by means of the microdisk diffusion method of Kirby-Bauer. These determinations were done according the NCCLS standards.

Results and discussion: Ninety-six *Salmonella* isolates were identified out of 3667 examined samples. When several samples in a given farm yielded the same serotype, only isolates having different phenotypic and antimicrobial susceptibility characteristics were considered. With this restriction, we considered to have 62 different strains belonging to 17 serotypes. The most common serotype was

Anatum (16.1 %, 9 farms) followed by Rissen (14.5 %, 7 farms), Typhimurium (11.3 %, 7 farms), Derby (9.7 %, 3 farms), Tilburg (8.1 %, 2 farms), Goldcoast (8.1 %, 1 farm), Typhimurium variant 4,5,12:i:- (6.5 %, 4 farms). Serotypes Bovismorbificans, Bredeney, Diarizonae, Grumpensis, Infantis, Kapemba, Kedougou, Ohio, Seftenberg and Virchow were also isolated although less than three times each. Antimicrobial susceptibility analysis showed that the highest level of resistance was against tetracycline (68.8 %). Sulphonamides and their combination with trimethoprim were only active against 67.7 % and 53.1 % of the strains, respectively. Regarding β -lactams, 41.6 % of the strains were resistant to ampicillin and 18.7 % were resistant to the combination of amoxicillin and clavulanic acid. Finally, 17.7 % of strains were resistant to chloramphenicol. Sixty-two percent of the strains showed resistance to three or more antimicrobial agents and 46 % were resistant to five or more drugs. The maximum number of compounds to which one strain was resistant was 10 (corresponding to a 4,5,12:i:- strain. In addition 3 strains were resistant to eight or nine compounds. None of the strains was resistant to colistin or ceftriaxone and 12 strains were susceptible to all antimicrobial agents tested (serotypes Anatum, Goldcoast, Kapemba and Ohio). These results indicate that sub-clinical infection with *Salmonella* can be produced by multi-drug resistant strains. In addition, with very few exceptions, most of the detected serotypes were able to be resistant to more than three drugs. This fact suggests that antimicrobial resistance in *Salmonella* isolates from pigs is not an exclusive property of a given serotype, for instance Typhimurium, but a widespread characteristic. However, the wider spectrum of resistance is usually found in the 4,5,12:i:- variants of Typhimurium (De la Torre *et al.*, 2002). Taking together, these results show that antimicrobial resistance is a real problem in swine isolates of *Salmonella* and reinforce the notion that an improved knowledge of the impact of antimicrobial agents used in swine is required.

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EFFECT OF CHLORTETRACYCLINE ON SALMONELLA AND THE FECAL FLORA OF SWINE

PR 04

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Summary: The goals of this study were to determine the impact of sub-therapeutic chlortetracycline in market swine diets on 1) the prevalence and antimicrobial resistance of *Salmonella enterica* 2) antimicrobial resistance of the aerobic Gram negative fecal flora. There was no significant difference in the prevalence or antimicrobial resistance of *S. enterica* isolates. For the gram-negative fecal flora, there was a statistically significant difference ($p < 0.05$) between treatment groups for the frequency of antimicrobial resistance in the gram negative flora with pigs receiving chlortetracycline having a greater frequency of isolates resistant tetracycline, gentamicin, and ceftriaxone, and a lesser proportion of isolates resistant to ampicillin.

Introduction: Antimicrobial resistance among human pathogens poses a serious burden to public health, resulting in \$0.15 to \$3 billion in health costs annually in the US (Rubkin, 1998). Increasing concerns over the contribution of antimicrobial use in agriculture to AR in human pathogens and

recent suggestions that pigs reared in modern production systems do not reap the performance benefits of growth promotant antibiotics (Ice et al, 1999; Dritz et al., 2002) has increased the scrutiny over this antimicrobial use. In the US >60% of swine production units routinely incorporate growth promotants in the daily ration for pigs. (Anonymous, 2002) There is a paucity of controlled, prospective intervention studies in modern US commercial production settings to evaluate the impact of sub-therapeutic antimicrobial use on AR. The goal this study was to evaluate, in a controlled manner, the impact of sub-therapeutic antimicrobial use on AR in market age swine using *S. enterica* and the aerobic Gram-negative fecal flora as indicator organisms.

Methods: Within each of 3 farms, treatments were assigned to temporally matched finisher barn pairs based on pig placement dates. For each matched pair, a barn was assigned to the treatment (50g chlortetracycline/ton of feed) or control (no antibiotics in the feed). A total of 22 barns were enrolled. Fecal samples were collected from 96 individual pigs per barn prior to slaughter. All samples were cultured for *S. enterica*, and isolates were tested for resistance to 17 antimicrobials. For the gram-negative fecal flora, 48 individual 1g fecal samples per barn were serially diluted (2, 10-fold dilutions) and plated onto MacConkey agar. One hundred colonies per sample were selected to create a master plate for replica plating. Master plates were replicated onto 5 agar plates each containing ampicillin, ceftriaxone, gentamicin, or tetracycline at NCCLS breakpoint concentrations. Comparison of *Salmonella* prevalence between treatment and non-treatment groups was conducted using Wilcoxon Signed Rank test (SPSS 11.5, SPSS, Inc). A binary logistic regression model (SPSS 11.5, SPSS, Inc) was initially constructed with individual colony resistance as the dependent variable and treatment and farm and an interaction term entered as independent variables. The final model was then analyzed in MLWLN 1.1 to assess the contribution of clustering to variance components.

Results: The overall prevalence of *S. enterica* was low (<1.0%). *Salmonella* were isolated from only one temporally matched barn pair, with 1.3% prevalence in the treatment barn and 0.09% prevalence in the control barn. For the gram-negative fecal flora, there was a statistically significant difference ($p < 0.05$) between treatment groups for the frequency of antimicrobial resistance in the gram negative flora with pigs receiving chlortetracycline having a greater frequency of isolates resistant tetracycline, gentamicin, and ceftriaxone, and a lesser proportion of isolates resistant to ampicillin (Table 1). Resistance to >1 antibiotics was more common in the treatment groups (29.0% and 18.2% for treatment and control respectively, $p < 0.05$). Results of multi-level modelling are pending. Preliminary results suggest that short-term changes in chlortetracycline selection pressure do not alter *S. enterica* prevalence and resistance, but are associated with statistically significant changes in the frequency of resistance in the fecal flora.

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Table 1. Proportion of aerobic gram-negative fecal flora isolates resistant to each of 4 antimicrobials in pigs receiving chlortetracycline and pigs not receiving chlortetracycline.

| Antimicrobial | Percent of Gram-negative isolates resistant (%) | |
|---------------------|---|--------------------------------|
| | Pigs fed chlortetracycline (50g/ton) | Pigs not fed chlortetracycline |
| ampicillin | 16.2 | 20.1 |
| ceftriaxone | 0.3 | 0.7 |
| gentamycin | 1.6 | 1.7 |
| tetracycline | 84.3 | 97.5 |

ANTIMICROBIAL RESISTANCE OF *Salmonella* ISOLATES FROM SWINE

PR 05

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Summary: The aim of this study was to report the antimicrobial resistance pattern of 27 *Salmonella* strains previously isolated from swine feces and lymph nodes collected in a slaughter-house in S. Paulo State, Brazil. The antibiotics tested were ampicillin (Amp), cephalothin (Cfl), ceftriaxone (Cro), streptomycin (Est), neomycin (Neo), gentamicin (Gen), amikacin (Ami), tobramycin (Tob), nalidixic acid (Nal), ciprofloxacin (Cip), norfloxacin (Nor), tetracycline (Tet), sulphazotrim (Sut) and chloramphenicol (Clo). Resistance to each antibiotic was determined by the Kirby-Bauer disk susceptibility test. Thirteen isolates (48.14%) were resistant to two or more antibiotics. Resistance to Tet was commonly seen among the isolates (55.5%). Total or intermediate resistance to Est (59.25%) were also commonly seen in the isolates. The high frequency of isolates resistant to Est and Tet may reinforce the possible link to the long-term therapeutic use of such drugs in pig production.

Keywords: Antibiotic, Kirby-Bauer, streptomycin, tetracycline, pigs

Introduction: Worldwide surveillance of *Salmonella enterica* serotyping and antimicrobial resistance is necessary in order to monitor emerging phenotypes. Furthermore, it permits to identify emerging clones and evaluate transmission of strains between regions (Petersen et al., 2002). The aim of this study was to determine the antimicrobial resistance patterns of some *Salmonella* strains isolated from swine in a commercial slaughterhouse located in S. Paulo Sate, Brazil.

Material and Methods: The antibiotics tested were extended spectrum penicillin (ampicillin-Amp), broad-spectrum cephalosporins (cephalothin-Cfl and ceftriaxone-Cro, aminoglycosides (streptomycin-Est, neomycin-Neo, gentamicin-Gen, amikacin-Ami and tobramycin-Tob), narrow-spectrum quinolone (nalidixic acid-Nal), broad-spectrum quinolones (ciprofloxacin-Cip, norfloxacin-Nor), tetracycline-Tet, sulphazotrim-Sut and chloramphenicol-Clo. Resistance to each antibiotic was determined by the Kirby-Bauer disk susceptibility test. The reference strains used were *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853. After the incubation period, the strains were finally interpreted as resistant, intermediate or susceptible to each antibiotic.

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Results: The antimicrobial resistance patterns are shown in Table 1. Thirteen isolates (48.14%) were resistant to two or more antimicrobial agents. Resistance to tetracycline was commonly seen among the isolates (55.5%). Resistance to Cip, Amp, Gent, Cfl, Cro, Ami, Nor, Tob and Neo were not detected. Total or intermediate resistance to Est (59.25%) were also commonly seen in the isolates. The resistance pattern CloEstTetSut was seen in seven *Salmonella* Typhimurium strains.

Discussion: The high resistance rates to tetracycline and streptomycin are in accordance with previous report on resistance of *Salmonella* isolates from swine in Brazil (Oliveira et al, 2002). Additionally, high frequencies of isolates resistant to tetracycline have been also detected in *Salmonella* isolates of swine origin collected in other countries (Gebreyes et al, 2000). In the USA, 84.2% of *Salmonella* strains isolated from swine were found to be resistant to tetracycline. It is likely that a link might exist between the long-term therapeutic use of certain antibiotics and the high number of isolates showing resistance patterns to them.

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Table 1: Antimicrobial resistance patterns from 27 samples comprised by 8 serotypes of *Salmonella* isolated from pig feces and lymph nodes of slaughter-age pigs from São Paulo State, Brazil. Ampicillin (AMP), cephalothin (CPT), cephaltriaxone (CRO), streptomycin (EST), neomycin (NEO), gentamycin (GEN), amikacin (AMI), tobramycin (TOB), nalidixic acid (NAL), ciprofloxacin (CIP), norfloxacin (NOR), tetracycline (TET), sulphazotrim (SUT) and chloramphenicol (CLO);

R = resistance; I=intermediate resistance.

| Antibiotic | <i>Salmonella enterica</i> serotypes | | | | | | | | |
|------------|--------------------------------------|--------------------|------------|---------------|-----------|-----------------|-----------------|------------------|---|
| | Mbandaka (3) | Schwarzengrund (4) | London (4) | Lexington (1) | Hadar (1) | Typhimurium (8) | Senftenberg (3) | S. 1,3,9:-:- (3) | |
| CIP | R | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | I | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| AMP | R | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | I | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| GEN | R | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | I | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| CLO | R | 0 | 0 | 0 | 1 | 1 | 7 | 0 | 1 |
| | I | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| EST | R | 0 | 0 | 0 | 1 | 1 | 8 | 3 | 1 |
| | I | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 2 |
| NAL | R | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| | I | 2 | 0 | 0 | 0 | 0 | 7 | 0 | 1 |
| CFL | R | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | I | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| CRO | R | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | I | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| SUT | R | 0 | 0 | 0 | 1 | 1 | 8 | 1 | 2 |
| | I | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| TET | R | 2 | 0 | 0 | 1 | 1 | 8 | 0 | 3 |
| | I | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| AMI | R | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | I | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| NOR | R | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | I | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| TOB | R | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | I | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| NO | R | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | I | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

Antimicrobial resistance of *Salmonella* strains isolated from pork products

PR 06

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Summary: The objective of this study was to assess the antibiotic resistance pattern in *Salmonella* strains isolated from sausages and pork cuts processed in the same slaughterhouse in Rio Grande do Sul, Brazil. The agar diffusion method was used to test ten different serovars isolated from sausages (n= 127) or from pork cuts (n=62) against 14 antimicrobial drugs. Resistance to sulphonamide (54.0%), tetracycline (35.4%), streptomycin (31.2%), nalidixic acid (28.0%), sulphametoazole/trimethoprin (24.9%), neomycin (22.2%), chloramphenicol (22.2%), ampicillin (20.6%), tobramycin (19.6%), cefaclor (14.3%), gentamicin (9.5%), amoxicillin/clavulanic acid (5.8%), amikacin (4.2%) and ciprofloxacin (2.1%) was observed. The multiresistance pattern (resistance against at least four antibiotics) was found in 39% of *Salmonella* strains. The multiresistance pattern was more common in pork cut isolates (62.9%) than in sausage isolates (27.5%). Strains of *S. Bredeney* and *S. Panama*, the two most isolated serovars in this study, showed the highest number of multiresistant strains (39.3% and 42.5%, respectively).

Keywords: swine, sausage, pork cuts, multiresistant.

Introduction: The use of antimicrobial drugs in therapeutic and as growth promoters in swine production systems may lead to selection of resistant strains of pathogens, including *Salmonella*, which can be transmitted to humans through the food chain (Bahson and Fedorka-Cray, 1999). It is thus important to monitor the antibiotic resistance pattern of foodborne pathogen strains isolated from animal products. The presence of *Salmonella* sp. in pork products was previously detected in southern Brazil (Bandeira et al., 2002), justifying studies to assess the level of resistance to the antimicrobial agents currently in use.

Materials and Methods: One-hundred and eighty-nine (189) strains of *Salmonella* sp. isolated from sausages (n= 127) or from pork cuts (n=62) processed in a slaughter plant in Southern Brazil were included in the study. The isolates were serotyped in Fundação Instituto Oswaldo Cruz and kept frozen (at -20° C) in BHI broth with glycerol 20 %. Stock cultures were recovered in BHI broth and tested for resistance against 14 antimicrobial drugs using the agar diffusion method (Barry and Thornsbery, 1985).

Results: The highest level of resistance was found against sulfonamide (54.0%), tetracycline (35.4%), streptomycin (31,2%) and nalidixic acid (28,0%). Strains of serovars Bredeney and Panama were the most resistant (Table 1 and Table 2, respectively). The multiresistance pattern (resistance against at least four antibiotics) was found in 39% (74/189) of *Salmonella* strains. The multiresistance pattern was more common in pork cut isolates (39/62-62.9%) than in sausage isolates (35/127-27.5%). Strains of *S. Bredeney* and *S. Panama*, the two most isolated serovars in this study, showed the highest number of multiresistant strains (39.3% and 42.5%, respectively).

Discussion: The highest level of resistance was found against antimicrobial agents frequently used in animal husbandry, which is in accordance with findings already established in other studies (Bahson and Fedorka-Cray, 1999; Fedorka-Cray et al., 1999; Harvey et al., 1999). The resistance pattern varied according to origin and serovar of the *Salmonella* isolates. Differences in type of antimicrobial drugs used for animal treatment in each herds can explain the high diversity in the resistance pattern found. The high number of multiresistant strains found in the present study points to the need of

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further monitoring of the antimicrobial usage on farms, to control the hazard of selection and transmission of resistant zoonotic strains.

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TABLE 1: Antimicrobial resistance pattern of *Salmonella* strains isolated from sausage in Rio Grande do Sul, Brazil.

| Serovar | Number of resistant strains | | | | | | | | | | | | | | |
|-----------------------|-----------------------------|----------|----------|-----------|----------|----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|
| | N | AC | AM | AP | CF | CI | CL | ES | GE | NA | NE | SL | ST | TE | TB |
| Agona | 1 | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Bredeney | 43 | 4 | 1 | 17 | 3 | 1 | 16 | 15 | 1 | 4 | 11 | 24 | 21 | 17 | 4 |
| Derby | 7 | - | 2 | 1 | - | - | 1 | 3 | - | 1 | 1 | 1 | 1 | 5 | - |
| Enteritidis | 2 | - | - | - | - | - | 1 | - | - | 1 | - | 1 | - | 1 | - |
| Mbandaka | 11 | 1 | - | - | - | - | - | 2 | - | - | - | 5 | 2 | 1 | - |
| Minnesota | 2 | - | - | 1 | - | - | 1 | 1 | - | - | - | 2 | 1 | 1 | - |
| Panama | 16 | - | - | 4 | 2 | - | 3 | 2 | - | 7 | 3 | 5 | 3 | 4 | 1 |
| Saint-paul | 28 | 1 | - | 1 | 2 | - | 2 | 2 | 9 | 2 | - | 3 | 1 | 2 | 19 |
| <i>Salmonella</i> sp. | 3 | - | - | 1 | 1 | 1 | - | 1 | - | 1 | 1 | 1 | 1 | 1 | 1 |
| Typhimurium | 14 | 1 | - | 3 | - | - | 7 | 6 | 1 | 7 | 1 | 10 | 7 | 11 | 2 |
| Total | 127 | 7 | 3 | 28 | 8 | 2 | 31 | 32 | 11 | 23 | 17 | 52 | 37 | 43 | 27 |

N: number of tested strains

AC: amoxicilin/clavulanic acid; AM: amikacin; AP: ampicillin; CF: cefaclor; CI: ciprofloxacin; CL: chloramphenicol; ES: streptomycin; GE: gentamicin; NA: nalidixic acid; NE: neomycin; SL: sulfonamide; ST: sulfamethoxazole/trimethoprin; TE: tetracycline; TB: tobramycin.

TABLE 2: Antimicrobial resistance pattern of *Salmonella* strains isolated from pork cuts in Rio Grande do Sul, Brazil.

| Serovar | Number of resistant strains | | | | | | | | | | | | | | |
|-----------------------|-----------------------------|----------|----------|-----------|-----------|----------|-----------|-----------|----------|-----------|-----------|-----------|-----------|-----------|-----------|
| | N | AC | AM | AP | CF | CI | CL | ES | GE | NA | NE | SL | ST | TE | TB |
| Bredeney | 13 | - | 1 | 3 | 5 | - | 4 | 3 | - | 6 | 3 | 10 | 2 | 3 | 1 |
| Derby | 4 | - | 1 | 1 | - | - | - | 4 | - | 1 | 4 | 3 | - | 4 | - |
| Mbandaka | 3 | 1 | - | - | 2 | - | - | 3 | 1 | 2 | - | 2 | - | 2 | 2 |
| Minnesota | 4 | - | - | - | - | - | 2 | 2 | 1 | 1 | 2 | 3 | 1 | 1 | - |
| Ohio | 2 | - | - | - | - | - | - | - | - | 1 | 2 | - | - | 1 | 1 |
| Panama | 24 | - | 1 | 4 | 5 | 1 | 1 | 22 | 5 | 11 | 12 | 20 | 2 | 3 | 3 |
| <i>Salmonella</i> sp. | 8 | 2 | 1 | 3 | 5 | - | 4 | 3 | - | 4 | 3 | 5 | 3 | 6 | 2 |
| Typhimurium | 4 | 1 | 1 | - | 2 | 1 | - | - | - | 8 | - | 3 | 2 | 4 | 1 |
| Total | 62 | 4 | 5 | 11 | 19 | 2 | 11 | 27 | 7 | 30 | 25 | 50 | 10 | 24 | 10 |

N: number of tested strains

AC: amoxicillin/clavulanic acid; AM: amikacin; AP: ampicillin; CF: cefaclor; CI: ciprofloxacin; CL: chloramphenicol; ES: streptomycin; GE: gentamicin; NA: nalidixic acid; NE: neomycin; SL: sulfonamide; ST: sulfamethoxazole/trimethoprin; TE: tetracycline; TB: tobramycin.

Antibiotic resistance of *Salmonella* strains isolated from pig slurry.

PR 08

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Summary: The survival of *Salmonella* sp. in pig slurry submitted to treatment in a stabilization ponds system from a pig breeding farm in southern Brazil were evaluated. The stabilization ponds system proved to be efficient, reducing 93% of the *Salmonella* sp. population present in the slurry. The resistance pattern of 161 *Salmonella* Typhimurium strains isolated throughout the system were evaluated against 14 antibiotics, using the agar diffusion method. *Salmonella* strains were resistant to sulfonamide (100%), tetracycline (99.4%), streptomycin (90.1%), sulfamethoxazole/ trimethoprin (84.5%), nalidixic acid (77.6%), ampicillin (76.4%), chloramphenicol (29.2%), cefaclor (25.5%), tobramycin (13.7%), gentamicin (6.2%), amoxicillin/clavulanic acid (5%), neomycin (5%), and amikacin (3.7%). All tested *Salmonella* strains were sensitive to ciprofloxacin. Most strains of *Salmonella* (94.5%) were resistant to four or more antibiotics. The multi-resistance pattern was found in strains isolated from all sampled points and were highly variable throughout the system.

Keywords: *Salmonella* Typhimurium; antibiotic resistance; pig slurry; stabilization pond system.

Introduction: Wastewater treatment facilities have been implicated as potential sites for the selection of multiresistant enteric strains (Taher et al., 1987). On the other hand, aerobic and anaerobic treatment systems proved to be efficient for the elimination of *Salmonella* present in pig slurry (Juris et al., 1995). The aim of the present study was to determine the presence of *Salmonella* sp. throughout stabilization lagoons used for the pig slurry treatment, as well as to compare the resistance profile of strains isolated from different points of the treatment plant.

Materials and Methods: Twenty samplings were conducted, about two times a month, in seven points throughout a system of serial lagoons located in southern Brazil. The system was composed by a storage tank (P1), a sludge tank (PA), a sedimentation tank (P3), two anaerobic lagoons (P9), one facultative lagoon (P10), one aerated lagoon (P11) and three aerobic ponds (P14). The isolation of *Salmonella* sp. was conducted as described by Michael (2002). Isolates were tested for resistance against 14 antibiotics, using the agar diffusion method (Barry & Thornsberry, 1985). For the calculation of the multiple antibiotic resistance (MAR) index proposed by Krumperman (1983), *Salmonella* strains were divided in three groups, according to the site of isolation: S1 (PA, P1 and P3, n = 138), S2 (P9, n = 15) and S3 (P10, P11 and P14, n = 11).

Results: *Salmonella* was isolated from 13/20 samples taken from the affluent (P1) of the system and from only one effluent sample (P14), indicating 93% of *Salmonella* population reduction during the treatment. Most *Salmonella* isolates (161/163) belonged to serovar Typhimurium. These strains were resistant to sulfonamide (100%), tetracycline (99.4%), sulfamethoxazole/trimethoprin (84.5%), ampicillin (76.4%), cloramphenicol (29.2%), streptomycin (90.1%), nalidixic acid (77.6%), tobramycin (13.7%), neomycin (5%), amikacin (3.7%), cefaclor (25.5%), gentamicin (6.2%) and amoxicilin/ clavulanic acid (5%). Strains showed between two and eleven resistance markers, being the most part of them (30.4%) resistant against six antibiotics. No significant difference was found among MAR indices of different groups (S1=0.43; S2=0.46 and S3=0.41). Fifty seven different resistance profiles were found, being the most frequent profile (ap-c-e-na-su-ti) identified in 38 (23.6%) strains. However, 28.6% of the identified profiles were represented by only one strain.

Discussion: In the present study, the solid phase separation (P1, PA and P3) proved to be inefficient for *Salmonella* elimination. The reduction of *Salmonella* populations started to be observed only after the anaerobic (P9) and facultative lagoons (P10), in agreement with previous studies (Hainonen-Tanski, 1998). As previously described (Bahnsen and Fedorka-Cray, 1999), the highest level of resistance was found against antimicrobial agents used on the farm. Although the selection of resistant strains during the slurry treatment (Morozzi et al., 1986) was described, in the present study no differences on the resistance profile could be observed among strains isolated from both affluent and effluent. According to Krumpermann (1983), MAR indices greater than 0.2 classify a contamination source as of high-risk. Thus, the multiple antibiotic resistance indices showed by *Salmonella* strains could be considered another potential hazard of these strains for the environment.

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Resistance to apramycin of *Salmonella* and *E.coli* isolated from swine.

PR 09

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Summary: The aim of this study was to determine the prevalence of aminoglycosides antibiotic resistance in *Salmonella* spp. and *E. coli* strains. 32 *E. coli*, and 47 *Salmonella* spp., isolated from cases of enteritis in growers and fatteners from 1998 to 2002 in Umbria and Marche regions, were tested. Susceptibility to gentamicin, tobramycin and streptomycin was determined by Kirby-Bauer method, apramycin by microdilution method. 92,4 % of the strains tested were susceptible to apramycin, 77,2 % to gentamicin, 67,1 % to tobramycin and 35,4 % to streptomycin. A positive statistical association between gentamicin and apramycin (RR = 7,63; p = 0,014), tobramycin and apramycin (RR = 9,22; p = 0,027) was demonstrated. There is no difference between the association apramycin-streptomycin, suggesting a mechanism of resistance related to the presence of the aminoglycoside acetyltransferase IV enzyme. The trend based on estimated OR from the resistance of the strains for every year considered was significant (p = 0,00049), showing a progressive decrease from 1998 (OR = 1) to 2002 (OR = 0,3).

Keywords: aminoglycosides, enteritis, gentamicin, tobramycin, streptomycin.

Introduction: Apramycin, an aminoglycoside antibiotic has been used in veterinary medicine since 1980, in oral treatment of Gram negative bacterial enteritis of swine (Johnson et al., 1994). The main objective of this study was to determine the prevalence of antibiotic resistance among strains of *Salmonella* spp. and *E.coli* isolates from cases of enteritis in growers and fatteners in Umbria and Marche regions. Furthermore, the relationship between susceptibility to apramycin and related aminoglycosides was evaluated. Finally, trend of resistance to these aminoglycosides from 1998 to 2002 was investigated.

Materials and methods: *Salmonella* spp. and *E.coli* isolates were identified as described elsewhere (Quinn et al., 1999); *Salmonella* spp. strains were serotyped according to Popoff (Popoff et al., 1997). MIC were performed using the microdilution method according to the National Committee for Clinical Laboratory Standards document for veterinary antimicrobial susceptibility tests (NCCLS, 1998). The dilutions ranged from 0,25 mg / ml to 128 mg / ml. Apramycin was kindly provided by EliLilly. The interpretative criteria used for apramycin were based on previous report (Prescott et al., 2000): strains with MIC \leq 16 mg / ml were regarded as susceptible. Susceptibility to gentamicin, tobramycin and streptomycin was determined by the Kirby-Bauer test using antimicrobial containing disks (Oxoid) according to the NCCLS document described above. The hypothesis of association between the resistance to apramycin and others aminoglycosides was tested using the Fisher's exact test. Values of p less than 0,05 were considered significant. The measure of this association was expressed by the relative risk (RR). The chi square value for the trend and the odds ratio were used to verify the resistance of the strain to aminoglycosides for every year considered.

Results: 79 strains, 32 belonging to *E. coli* species, and 47 to *Salmonella* spp., were used in this study. Among *Salmonella* spp., the predominant serovar was Typhimurium (69 %), followed by Panama, (7 %), Seftemberg (4 %), Anatum (4 %) and Bredney (4 %), while other serovars did not exceed 2 %. In figure 1 frequency (percentage) of strains related to MIC values are shown. The interpretative criteria used for apramycin were based on previous report (Prescott et al., 2000): strains with MIC \leq 16 mg / ml were regarded as susceptible: so, 92,4 % of the strains tested were classified as susceptible. A

high percentage of strains tested were susceptible to gentamicin (77,2 %); tobramycin (67,1 %), but not to streptomycin (35,4 %). The relationship between the resistance of isolates to apramycin and others antibiotics is shown in table 1. The trend based on odds ratio (OR) estimated from the resistance to all the aminoglycosides for every year considered is shown in figure 2.

Figure 1 Distribution of strains related to MIC

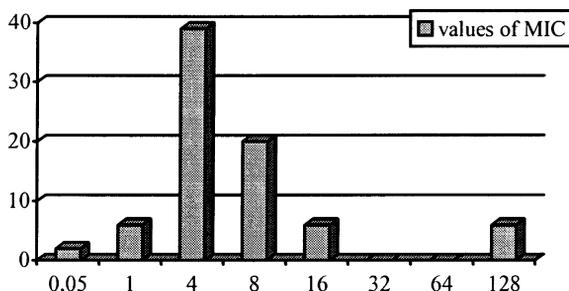
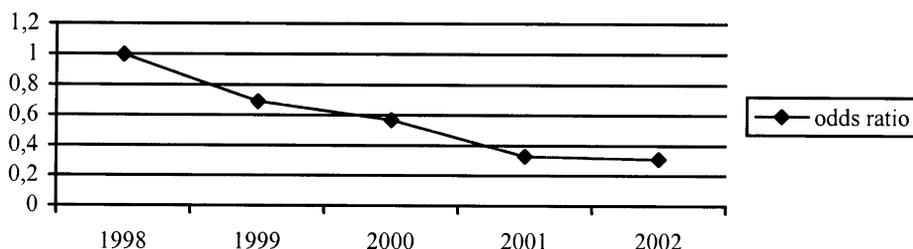


Table 1 Results of univariate analysis

| | RR | Lower limit | Upper limit | p |
|--------------|------|-------------|-------------|-------|
| Gentamicin | 7,63 | 1,53 | 37,98 | 0,014 |
| Streptomycin | - | - | - | 0,122 |
| Tobramycin | 9,22 | 1,09 | 78 | 0,027 |

Figure 2 Trend of strain resistant of apramycin during 5 years



Discussion and Conclusions: 92,4 % of the strains tested were classified as susceptible to apramycin. These results are consistent with previous reports, as resistance to apramycin is rare among Gram-negative bacteria. Strains were either clearly susceptible (< 8mg / ml) or resistant (> 128 mg / ml) none of the strains tested showed values between 16 to 64 mg / ml. Such distribution, rather than a normal, seems the bimodal distribution typical for most antibiotics and for most pathogens (Prescott et al., 2000). A positive statistical association between gentamicin and apramycin (RR = 7,63; p = 0,014), tobramycin and apramycin (RR = 9,22; p = 0,027) was demonstrated. Since aminoglycoside acetyltransferase IV degrade apramycin, gentamicin and tobramycin, but not streptomycin, these results may suggest a mechanism of resistance related to this enzyme. The trend of the resistance of strains to aminoglycosides for every year considered is significant (p = 0,00049) showing a progressive decrease from 1998 to 2002. These data need further investigations but these results could also be explained by a decreased employment of aminoglycosides in feed.

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Survival of *Salmonella* and *Escherichia coli* in pig slurry: simulation of decay **PMP 01**

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Summary: Spreading of slurry infected with multi-resistant *Salmonella* Typhimurium DT104 (MRDT104) on arable land might constitute a risk of transmission to wildlife. To estimate survival time on farmland, we modeled the bacterial decay based on *Escherichia coli* data from a plot study carried out in spring 2002 in Denmark. Time until undetectable levels were modeled under different scenarios: 1) *E. coli* in swine slurry, 2) *Salmonella* in slurry from clinically infected swineherds, and 3) MRDT104 in slurry from sub-clinically infected swineherds. A log-linear model extended with time² and time³ was used to describe bacterial decay. For scenarios 2 and 3, we assumed that the level of bacteria in the slurry would be log 4.0 cfu/g and log 3.4 cfu/g, respectively, and a similar effect of spreading and decimation to that of *E. coli*. Hereby, it was estimated that *Salmonella* counts fell below detectable levels after 10 and 5 days, respectively.

Keywords: microbial ecology, transmission, multi-resistant *Salmonella* Typhimurium DT104, environmental persistence, decimation

Introduction: Spreading of slurry infected with multi-resistant *Salmonella* Typhimurium DT104 (MRDT104) on arable land has been considered a potential hazard for transmission to wildlife. Therefore, spreading has been restricted for herds positive to MRDT104. Our aim was to model decay of *Escherichia coli* and *Salmonella* after spreading of contaminated slurry on farmland, and to estimate the survival time.

Materials and Methods: The modeling was based on *Escherichia coli* data from a plot study carried out in spring 2002 in Denmark. Here, *E. coli* was measured quantitatively on day 0, 7, 14, 21, and 28 after application on soil using 4 different methods (see Boes & Alban, in this issue). *Salmonella* was not detected when slurry was ploughed in, and hence, these data were not used for the modeling. Data from the three remaining application methods (harrowed only, slurry injection, and hose application) were used.

We were interested in estimating the time from disposal until undetectable levels under different scenarios: 1) *E. coli* in swine slurry, 2) *Salmonella* in slurry from clinically infected swineherds, and 3) MRDT104 in slurry from sub-clinically infected swineherds.

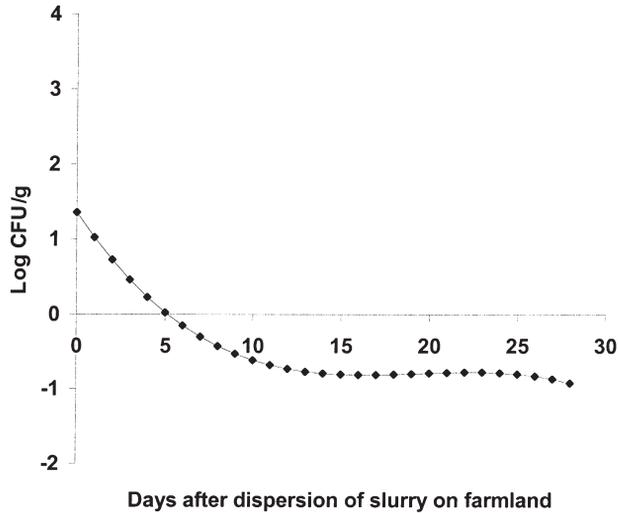


Fig. 1: Modeled decay of multi-resistant *Salmonella Typhimurium* DT104 in slurry from sub-clinically infected swineherds based on *E. coli* data from a Danish plot study, carried out in 2002. Detection level for *Salmonella* was 1 cfu/g = 0 Log.

A log-linear model extended with time² and time³ was used to describe bacterial decay. The detection level was log 1 cfu/g for *E. coli* and log 0 cfu/g for *Salmonella* (enrichment phase included in laboratory procedure).

For scenarios 2 and 3, we assumed that the level of bacteria in the slurry tank would be log 4.0 cfu/g and log 3.4 cfu/g, respectively. Furthermore, we assumed a similar effect of spreading and decimation to that of *E. coli*.

Results: The following model was found to be the best at explaining the decay of *E. coli*: $\log(E. coli) = 3.2150 - 0.3508 * \text{time} + 0.0186 * \text{time}^2 - 0.0003 * \text{time}^3$ ($r^2 = 81\%$). According to this model, *E. coli* levels reached the detection limit after 15 days.

For *Salmonella* (scenario 2 and 3) it was estimated that the bacterial counts fell below detectable levels after 10 and 5 days (Fig. 1), respectively.

Discussion: *E. coli* was used instead of *Salmonella*, because it occurs in much higher numbers, and its decimation time (T_{90}) is comparable. T_{90} for *E. coli* and *Salmonella* in our model were similar to T_{90} obtained from observational studies. The level of *E. coli* was representative for swine slurry, as were the assumed levels of *Salmonella* in slurry from clinically and sub-clinically infected swineherds.

Our findings are in agreement with results from observational studies, e.g. the plot study, where *Salmonella* was detected in soil samples until day 7, where 1 sample out of 32 was positive (Boes & Alban, in this issue).

The results are contrary to results from experimental studies. This might be because much higher bacteriological levels are used in experimental studies. Furthermore, in experimental set-ups it is difficult to capture the multi-factorial nature of the biological processes influencing bacterial survival. Finally, our study estimated the average decline of *Salmonella*, without aiming to show when all viable counts had truly disappeared.

Conclusion: We conclude that MRDT104 levels in slurry from sub-clinically infected swineherds are below detectable levels less than 10 days after application on farmland. The low detection limit for *Salmonella* implies, that hereafter, only a negligible concentration of MRDT104 is left in the soil, lowering

the risk of transmission to wildlife. Until the 10 days have passed, MRDT104 bacteria are present, and as such constitute a potential hazard. However, as it is not allowed to spread slurry from MRDT104 infected herds on pasture or ready-to-eat vegetables, there is no direct exposure to grazing stock or humans.

Risk analysis of *Bacillus* spp. isolated from cured pork sausages

PMP 02

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Summary: This study was undertaken to acquire information about the toxigenic potential of *Bacillus* strains isolated from eight cured pork sausages obtained from traditional or industrial processings. The application of RAPD-PCR protocols made it possible to identify 52 different biotypes among 220 heat-resistant Gram-positive endospore-forming colonies. The sequence analysis of the 5' region of 16S rDNA revealed that 36 strains belonged to *B. subtilis* and 16 to *B. pumilus* species. No strains belonging to *B. cereus* species were isolated from the cured sausages analysed. The toxigenic potential of these strains was assayed by PCR analysis and physiological tests to identify the most important *B. cereus* toxins and virulence factors. No specific PCR fragment was obtained from any of the strains; however, some of them were found positive for hemolytic and lecithinase activity. These preliminary results reassure about the microbiological risk related to the presence of pathogenic *Bacillus* strains in cured pork sausages analysed even though the hemolytic and lecithinase activities found in some strains suggest that more in-depth analyses need to be carried out.

Keywords: PCR, toxins, virulence factors, *B. cereus*, cured meat products

Introduction: A wide variety of microorganisms such as, lactic acid bacteria (LAB), *Staphylococcus*, *Kocuria* and *Bacillus* are involved in meat fermentation. Most cases of food poisoning attributed to *Bacillus* species are associated with *B. cereus*; this bacterium is known to cause a variety of foodborne disorders characterized by either diarrhea or emesis. Lately, other *Bacillus* species have been gaining recognition as organisms relevant in causing food poisoning, with recent epidemiological evidence linking *B. subtilis*, *B. pumilus*, *B. licheniformis*, and *B. thuringensis* with incidents of foodborne illness. Evaluation of toxin gene presence and expression in *Bacillus* spp. other than *B. cereus* has not been thoroughly investigated. The survival of *Bacillus* strains through meat processing leads to suppose that potentially pathogenic ones could be present in cured sausages. For these reasons we analyzed *Bacillus* strains isolated from industrial and traditional cured pork sausages to gain insight into their potential role in foodborne infections.

Material and Methods: Eight sausage samples were collected from the local market; the sausage casing was removed aseptically and 20 g sample from the central portion of each sausage was homogenized (10 % w/w) in a saline solution. Five milliliters of cell suspension were pasteurized at 80 °C for 10 min and then cooled to room temperature. Serial decimal dilutions in 0.1 % peptone water were poured onto non-selective tryptose soy agar plates (Oxoid, Basingstoke, UK). Aerobic mesophilic counts were determined after incubation at 30 °C for 72 h. Thirty colonies from each sample were collected and analyzed for Gram stain, cell morphology, presence of endospores and catalase reaction. The genomic DNA of each isolate was extracted with DNA Purification Kit (Promega, UK). The isolates were biotyped and taxonomically identified by using a two-step RAPD-PCR protocol and 16S rDNA sequencing (Baruzzi et al., 2000). By means of PCR assays, the strains were analysed for most

important *B. cereus* virulence factors: enterotoxins FM/S (*entFM*), T (*bceT*), and NHE (*nheB*), HBL, a three-component hemolysin with hemolytic and dermonecrotic activities (*hbl-D*), sphingomyelinase (*sph*), and phosphatidylinositol specific phospholipases (*pipIc*) (Ghelardi et al., 2002).

All the strains were tested for hemolytic activity after growth on blood agar plates (Merck, Darmstadt, Germany) containing 5 % sheep blood. Lecithinase-positive strains produced an halo around the colonies grown onto nutrient agar supplemented with 8 % egg yolk emulsion (Oxoid).

Results: RAPD-PCR analysis showed 52 different fingerprints from 220 colonies. The 52 biotypes identified by means 16S rDNA sequencing belonged to *B. subtilis* and *B. pumilus* species with 36 and 16 strains, respectively. No *B. cereus* strain was isolated from cured pork sausages analyzed. The distribution of total viable cell counts of *B. subtilis* and *B. pumilus* strains from each sample is shown in Figure 1.

No PCR fragment related to *B. cereus* virulence factors was obtained from *B. subtilis* and *B. pumilus* strains from cured sausages. The sequence analyses of DNA fragments amplified from positive control strains (*B. cereus* type strains DSM4312 and DSM4313 and *B. cereus* BAC1 dairy isolate) showed that they exhibited a high degree of identity (97-100 %) with the *B. cereus* virulence genes.

The results relative to hemolytic activity showed that 15 out of 16 *B. pumilus* strains were positive whereas only two *B. subtilis* strains produced a weak halo of hemolysis after 48 h growth. Lecithinase activity was developed by 72 % *B. subtilis* and one *B. pumilus* strains.

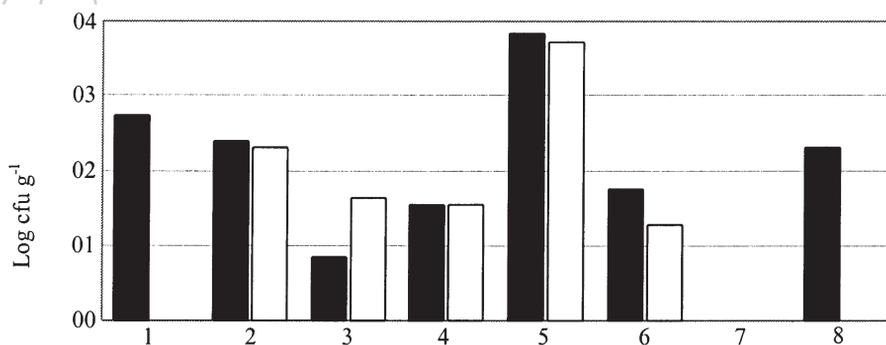


Fig. 1 *Bacillus subtilis* and *B. pumilus* viable cell counts (black and white columns, respectively) from industrially (1-4) and traditionally (5-8) cured sausages

Discussion and Conclusions: This is a report of the preliminary results relative to the risk assessment of *Bacillus* spp. in cured pork sausages. The low levels of viable endospore counts in sausages (the minimal infective dose for the diarrhoeal type is assumed to be 100,000 *B. cereus* cells), the absence of *B. cereus* isolates and the absence of *B. cereus* virulence factors in *B. subtilis* and *B. pumilus* strains from traditional and industrial sausages seem to indicate that the samples analyzed do not pose any risk to consumer. Although the hemolytic and lecithinase activity could be considered normal metabolisms in cells from food matrix, more in-depth studies should be carried out to understand if new potential virulence factors are expressed in *B. pumilus* and *B. subtilis*.

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Peracute Infection of Swine With Salmonella

PMP 03

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Summary: It has recently been experimentally demonstrated that pigs exposed naturally to Salmonella on the floor of abattoir holding pens can become infected between two and six hours after being placed in the pens. In addition we have demonstrated that tonsillar tissue are almost immediately culture positive following such exposure under experimental conditions. The objective of this study was to determine the shortest amount of time necessary for infection of selected tissues and to determine if the tonsil served as a route for Salmonella entry into lymphoid tissues draining the tonsil. Forty-four Salmonella-negative, market age pigs (90 to 110 kg) were fasted overnight and exposed to approximately 2×10^6 *Salmonella enterica* serotype Typhimurium strain X4232 (nalidixic acid resistant). The bacteria were mixed with a fecal slurry and the slurry spread on the floor of the pens. Pigs were euthanized at 15, 30, 45, 60 and 120 minutes following initial exposure. Tonsil of the soft palate, medial retropharyngeal lymph node, ileocecal lymph node, a five centimeter section of the terminal ileum, cecal contents and 100 ml of blood were cultured for Salmonella. Strain X4232 was isolated from 98 % (43/44) of tonsils. Strain X4232 was isolated from the ileocecal lymph node within 45 minutes (2/9 pigs), terminal ileum within 15 minutes (1/9 pigs), cecal contents within 15 minutes (1/9 pigs), and blood within 45 minutes (1/9 pigs). Strain X4232 was not recovered from the medial retropharyngeal lymph node, indicating that the organism did not move rapidly into this node from the tonsil of the soft palate. Results of this study indicate that Salmonella can be recovered from selected tissues in market age swine in less than the normal two hour abattoir holding time.

Introduction: It has been demonstrated that a variety of Salmonella serotypes can be recovered from holding pen floors at abattoirs and that swine can become acutely infected with salmonellae during lairage at these facilities (Hurd, et al 2001a and b, McKean, et al. 2001). Strategies have been proposed to prevent or lessen this rapid infection including decreasing or totally eliminating the time pigs are held in lairage. Earlier work in our laboratory demonstrated that salmonellae can be recovered from the tonsil of the soft palate within two hours following oral exposure. The present study was conducted to determine if the tonsil could serve as a portal of entry for salmonella into pork and just how rapidly salmonellae could be found in intestinal sites and associated lymphoid tissues following oral exposure.

Methods: Forty-four pigs weighing 90 to 110 kg were purchased from a high-health status herd two weeks prior to challenge exposure. The facilities could accommodate a maximum of 12 pigs at a time and the experiment was repeated 4X. Fecal samples were obtained on all pigs on days -12, -5 and -3 and cultured for salmonella by the method of Hurd, et al 2001a. On day 0 all pigs were challenged orally with *Salmonella enterica* serotype Typhimurium strain X4232 (nalidixic acid resistant). The organism was grown overnight in tryptose broth and added to a slurry composed of 7 parts pig feces and 4 parts water. The slurry contained approximately 2×10^6 strain X4232 per ml and was spread on about 1 m^2 of the pen floor. Pigs were anesthetized, bled for culture, exsanguinated and necropsied at 15, 30, 45, 60 and 120 minutes following oral exposure to strain X4232. Tissues were collected aseptically and cultured for strain X4232 on nalidixic acid-containing media. Tissues sampled were 100 ml blood, tonsil of the soft palate, medial retropharyngeal and ileocecal lymph nodes, terminal ileum, and cecal contents. The blood was withdrawn from the anterior vena cava prior to exsanguination and was enriched in peptone water containing 1% SPS.

Results: Culture results from four trials were combined and are presented in Table 1. tonsillar tissues were uniformly culture positive for strain X4232 within 15 minutes of exposure to the organism via the pen floor. Retropharyngeal lymph nodes were negative on all pigs at all time points. Intestinal sites were culture positive in 2/9 pigs euthanized 15 minutes following exposure. Strain X4232 was recovered from the ileocecal lymph node in as little as 30 minutes following oral exposure (1/9 pigs).

Conclusions: Based on the results of this study, it does not appear that the tonsil of the soft palate is a major portal for peracute entry of salmonellae into draining lymphoid tissues. Also, salmonellae can be recovered from intestinal sites of pigs in as little as 15 minutes following oral exposure via the pen floor.

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Table 1. Salmonella recovery from tissues and intestinal contents. Pigs were euthanized and necropsied 15, 30, 45, 60 and 120 minutes following exposure. Values are the number of pigs positive for Salmonella vs the total number of pigs necropsied at each time point.

| Tissue | 15 min pos/total | 30 min pos/total | 45 min pos/total | 60 min pos/total | 120 min pos/total |
|--|---------------------|---------------------|---------------------|---------------------|----------------------|
| Tonsil of the soft palate | 9/9 | 9/9 | 9/9 | 8/9 | 4/4 |
| Medial retropharyngeal lymph node | 0/9 | 0/9 | 0/9 | 0/9 | 0/4 |
| Ileocecal lymph node | 0/9 | 1/9 | 1/9 | 0/9 | 1/4 |
| Distal ileum | 1/9 | 2/9 | 1/9 | 3/9 | 1/4 |
| Cecal contents | 1/9 | 2/9 | 0/9 | 1/9 | 1/4 |
| Blood | 0/9 | 0/9 | 1/9 | 0/9 | 0/4 |
| Pigs positive (excluding tonsil positives) | 2/9 | 2/9 | 2/9 | 3/9 | 2/4 |

KEYWORDS

| | | | |
|---|------------------------|------------------------------------|--------------------|
| Abattoir..... | O20, O49, O73 | Clustering..... | O17 |
| Acid..... | O45 | Coarse Grinding..... | O45 |
| Adaptive Resistance..... | PR1 | Coliforms..... | O47 |
| Aflp..... | O36 | Colistin..... | PR3 |
| Agar Dilution..... | O80, O81 | Colony Hybridization..... | O44 |
| Ag-ELISA..... | O15 | Comparison..... | O29, O31, PD2 |
| Agreement..... | PD8 | Contamination..... | PD2, PS3 |
| Aminoglycosides..... | PR9 | Control..... | O76 |
| Animal Feed..... | PD10 | Critical Points..... | PI5, PS2 |
| Animal Handling..... | O63 | Culture..... | O23 |
| Animal Health..... | PE5 | Cured Meat Products..... | MP2 |
| Antemortem Handling..... | O4, PS2 | Databank..... | O61 |
| Antibiotic..... | O79 | Decimation..... | MP1 |
| Antibiotic Resistance..... | O6, O22, PE1, PE4, PR8 | Decontamination..... | O61, PI2 |
| Antibiotics..... | PR1 | Dendogram..... | PE1 |
| Antimicrobial..... | O69, O74 | Detection..... | O28, PD5 |
| Antimicrobial Drugs..... | O68 | Dfd..... | PS2 |
| Antimicrobial Resistance..... | O21 | Diagnostics..... | PD3, O24 |
| Antimicrobial Use..... | O80 | DIG Nucleotide Probe..... | O44 |
| Antimicrobials..... | O67, PI4, PS3 | DNA Polymerase..... | PD10 |
| Api 20e..... | O31 | Drug Statistics..... | O69 |
| Apx Toxin..... | PD4 | Dt104..... | PM1 |
| Arcobacter Prevalence..... | O82 | Duration Of Infection..... | PE5 |
| Assurance Scheme..... | O63 | Dynamics..... | PE5 |
| Australia..... | O64 | E. Coli..... | O47 |
| Automation..... | O26 | E. Colo54i, Pigs..... | O54 |
| B. Cereus..... | MP2 | Economic Evaluation..... | O50 |
| Bacteria..... | PI1 | Elisa..... | O24, O49, PD4, PE6 |
| Bacterial Decay..... | O84 | Endemic..... | PE1 |
| Bacterial Genetics..... | O67 | Enrichment..... | O28, PD5 |
| Bacteriological..... | PE11 | Enterisol® SC-54..... | O76, O77 |
| Bacteriological Analysis..... | O1 | Enteritis..... | PR9 |
| Bacteriological Screening..... | O3 | Enterobacteriaceae..... | O58 |
| Bacteriology..... | O17, O33, O49 | Enterococcus Faecium/Faecalis..... | O37 |
| Barley..... | O45, O46 | Enterotoxigenic..... | O52 |
| Bedding..... | O83 | Environmental Persistence..... | MP1 |
| Belgium..... | O82 | Enzootic Pneumonia..... | PD3 |
| Bench Marking..... | PI5 | Epidemiology..... | O33, O48, O70 |
| Between-Herd Variability..... | O17 | Eradication..... | PD3 |
| Biocides..... | PR1 | Erm(B)..... | O65 |
| Biosecurity..... | O48, O55, O56 | Erythromycin..... | O66 |
| Botulism..... | O29 | Escherichia Coli O157..... | O37, O66 |
| Browning..... | PR4, PS4 | Excretion..... | O55, O56 |
| Caecal..... | O64 | Excretors..... | O13 |
| Campylobacter..... | O37, O43, O60 | Farm..... | O51 |
| Carcass..... | PS1 | Farm Level..... | O82 |
| Carcass Contamination..... | O49 | Farms..... | O13, PD8 |
| Carcasses And Organs Contamination..... | O12 | Farm-To-Fork..... | O39 |
| Carrier..... | O14, O41 | Fecespd6, PBS..... | PD6 |
| Catalonia..... | O14 | Feed..... | O42 |
| Cattle..... | O3 | Feed Intervention..... | O45 |
| Central Data Collection And Processing..... | O72 | Feeding Strategy..... | O46 |
| Certification..... | O75 | Field Trials..... | O52 |
| Chlorohexidine..... | O66 | Fige..... | PD7 |

KEYWORDS

| | | | |
|------------------------------|---|---|----------------|
| Fingerprint..... | O6, O79 | Longitudinal Study | PE11 |
| Fingerprinting..... | PR2 | Lost Days Of Work..... | O35 |
| Finisher Pigs..... | O45 | Lymph Nodes..... | PG1 |
| Fluoroquinolones..... | O80 | Macrolide Resistance | O65 |
| Food..... | PD11 | Mama Pcr..... | O81 |
| Food Policy..... | O63 | Mannan oligosaccharide..... | PI1 |
| Food Safety | O4, O6, O8, O9, O10, O11,O20, O34, O43, O50, O53, O73,O74, O77, O79, PE6, PE9 | Market Swine | O10 |
| Gastrointestinal Tract..... | O40, PI1 | Meat..... | O60 |
| Gene Expression..... | O29 | Meat Inspection | O19 |
| Genotyping..... | O26, PD10, PR2 | Meat Juice | PE6 |
| Gentamicin..... | PR9 | Meat Juice Antibodies..... | PE2 |
| Greece..... | PS3 | Meat Science..... | O4 |
| Grinding..... | O56 | Meat-Juice..... | O10 |
| Grow-Finish..... | PI4 | Media | O28 |
| Gyra..... | O81 | Medication Costs..... | O52 |
| Haccp..... | O61, O63 | Mesenterial Lymph Nodes..... | O17 |
| HACCP Methodology..... | O51 | Microbial Ecology..... | MP1 |
| Harrowing..... | O84 | Microbial Flora | O42 |
| Hazards..... | O51 | Minced Meat..... | O36 |
| Herd Level..... | PE2 | Mix-ELISA..... | O27 |
| Herd-Status..... | O33 | Mixing Groups | O12 |
| Hide Status..... | O58 | Molecular Methods..... | O29 |
| High Pressure..... | PI2 | Molecular Typing..... | PD7 |
| Hippuricase Gene..... | O44 | Monitoring..... | PD2 |
| Hose Application..... | O84 | Mortality | O52 |
| Human Disease..... | O21 | Multidrug Resistance..... | PR2 |
| Human Health..... | O34, O39 | Multiresistance..... | PR3 |
| Hygiene..... | O48 | Multiresistant..... | PR6 |
| Ig Class..... | O24 | Mycobacteriosis..... | O19, O83 |
| Immunoblot..... | PD4 | Mycobacterium Avium Complex..... | O83 |
| Incoming Animals..... | O56 | Nahms..... | O8, O9 |
| Indicators..... | O63 | Neonatal Piglet..... | O47 |
| Indole..... | O32 | Neurocysticercosis..... | O15 |
| Integrins..... | PR2 | Neurotoxin..... | O29 |
| Intervention..... | O74 | Odds Ratio..... | O27 |
| Intervention | O48 | On-Farm Epidemiology..... | O8, O9 |
| Intervention Strategies..... | PI5 | Organic Acids | O46 |
| Isolated..... | O70 | Organizational Structures of The Swine Industry...O72 | |
| Kappa..... | PD8 | Outbreak..... | PS1 |
| Lactating Sow..... | O47 | Outbreaks..... | O70 |
| Lactic Acid..... | O54 | Paramagnetic Beads | O36 |
| Lactobacilli..... | O47 | Pathogen..... | PP1 |
| Lactobacillus | O54, O40, PP1 | Pathogens Detection..... | PD11 |
| Lairage..... | O12, O20, O73 | Pcr..... | O20, O36, PMP2 |
| Lawsonia..... | PI4 | Pcr-Rflp | O22 |
| Lb-Bsi..... | PD6 | Pect | O83 |
| Lipopolysaccharides..... | PD12 | Pediococcus | O40 |
| Listeria..... | O58 | Pelleted..... | O56 |
| Listeria Monocytogenes | O37, O60, O62, PI2 | Performance..... | O77 |
| Listeria Spp..... | O62 | Persistence..... | PE4 |
| Liver White Spots..... | PE2 | Pfge..... | O20 |
| Livestock..... | O13 | Phage Type..... | O6 |
| | | Phagetype..... | O21 |
| | | Pig..... | O79 |

KEYWORDS

| | | | |
|----------------------------------|---|----------------------------|---|
| Pig Carcasses | O19 | Salmonella Load | PI5 |
| Pig Disease | PD3 | Salmonella Prevalence | O59 |
| Pig Faeces | O1 | Salmonella Serotypes | O68 |
| Pig Level | PE2 | Salmonella Shedding | PE10 |
| Pig Monocyte | O41 | Salmonella Typhimurium | O41, PR8 |
| Pig Slurry | PR8 | Salmonellae | O6 |
| Pig-Bacteria | O33 | Salmonella-Free | O55 |
| Pigs | O23, O28, O39, O40, O59, PD5 PI1, PP1, PR2, PS1, PS2 | Salmonellosis | PD7 |
| Plasmid Profile | PE4 | Sample Types | O29, O31 |
| Ploughing | O84 | Sampling | O61 |
| Point Mutations | O26 | Sanitation | O62 |
| Pooling | O1 | Sausage | PR6 |
| Porcine Faeces | O82 | Sensitivity | O1, O32, PD6 |
| Pork | O11, O23, O53, O59 O61, O62, PE9, PS1 | Sequencing | O81 |
| Pork Cuts | PR6 | Seroconversion | PD4 |
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