Foreword

Food safety is a continuum and effectively addressing food safety issues requires coordination throughout the food chain. Production at the farm level is the first link in the chain. The ecology of Salmonella in the farm environment is complex and multi-factorial. Because of the ubiquitous nature of Salmonella, on-farm control requires addressing reduction of levels in multiple areas. The dynamic interaction and enormity of the task can be visualized as:

The First International Symposium on the Ecology of Salmonella in Pork Production was organized to bring together researchers, industry, and government agencies to discuss current Salmonella research and identify research needs. The format of the Symposium was to provide an opportunity for presentations by industry and government agencies to explain their research needs and concerns followed by short research presentations from the research community. Break-out groups on the topics of pathogenesis/transmission, feed, and Hazard Analysis and Critical Control Point (HACCP) outlined future research areas.

We would like to thank our sponsors for their support and the National Animal Disease Center for the use of their facilities. Additionally, the Symposium received endorsement from the U.S. Animal Health Association's Feed Safety Committee.

Any errors in spelling or layout are the responsibility of the editors.

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The purpose of this meeting is to bring together as many investigators and parties interested in the field of salmonellosis in pigs to facilitate an open discussion of where we are and where we need to be going. While the title "Ecology of Salmonella in Pork Production" is all encompassing, all aspects of production impact on the quality of the product as it moves through to presentation at the table.

It is our responsibility, with today's limited resources, to obtain the maximum amount of useful information in a timely manner. The only way this can be accomplished is by sharing our knowledge and joining with one another to establish productive collaborations. As we openly share our data and ideas, and join with one another in future projects, the goal of the symposium will be met.
Swine *Salmonella* Research in the ARS

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Food safety remains an urgent concern for U.S. consumers. Although there is concern with chemical residues, mycotoxins and plant toxins, the greatest attention is given to microbiological pathogens. ARS has a broadly based food safety research program addressing both pre- and post-harvest opportunities for pathogen reduction. Procedures and practices to prevent pathogens, and in particular *Salmonella*, are needed to assure the safety of raw products because meat may not be cooked to recommended temperatures prior to consumption and because pathogens on raw product may be transferred to other foods that are eaten raw. ARS recognizes that the ecology of *Salmonella*, both inside the gastrointestinal tract and in the environment, is a critical research avenue because the population pressures affecting an organism must be understood in order to develop controls that are effective in a variety of settings and for the long term. Research at the National Animal Disease Center is the cornerstone of the ARS preharvest program to prevent *Salmonella* in swine.
FDA Food Safety Research and the Role of USDA in the Identification and Control of *Salmonella*

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**ARS Research Program**

The Agricultural Research Service (ARS) is the inhouse research arm of the Department of Agriculture, that is, ARS performs all the research for the Department, except that carried out by the Forest Service. In addition the agency meets major research needs of industry stakeholders. This approximately $700M, is spread over 7 different areas: soil, water and air; plant sciences, animal sciences, commodity conversion and delivery, human nutrition research, integration of agricultural systems, and finally agricultural information and library services. The National Agricultural Library has now been combined administratively with the ARS for about 2 years. These research programs are carried out in 106 research locations and 35 worksites.

The ARS food safety program is funded at approximately $45 M and is carried out in 15 different locations. Food safety research in the ARS provides the means to ensure that the food supply is safe for consumers and that food and feed meet foreign and domestic regulatory requirements. This research includes four major areas; that is, microbial pathogens, mycotoxins, toxins found in plants, and chemical contaminants. Pathogen control is the largest research area, and *Salmonella* spp. are the major pathogens of concern, although they are being challenged by others, such as *E. coli* 0157:H7.

Food safety pathogen control includes both preharvest (animal production) and postharvest (slaughter and processing) research. The preharvest research is carried out at Beltsville, Maryland; Athens, Georgia; College Station, Texas; Clay Center, Nebraska; and Ames, Iowa; and the post harvest pathogen research is located at Philadelphia, PA; Beltsville, MD; Athens, GA; Clay Center, NE; and Albany, CA. Just as with all of ARS research, producers, industrial stakeholders, regulatory agencies, and most importantly, American consumers all benefit from ARS pathogen reduction research.

The ARS pathogen control research program includes methodology, information for regulatory decisions, and interventions. **Methodology** is new, faster, less expensive, more accurate, more precisely defining methods that can be used by either regulatory laboratories and/or producers. It does include some studies of sampling protocols, which are becoming far more important recently with the imminent publication of the FSIS regulations requiring HACCP in all inspected establishments. **Information on which to base regulatory decisions** includes models of bacterial growth kinetics, inactivation and survival. This is the type of information that is vital to the risk assessment procedures carried out by the FSIS. Finally the **interventions** are a wide ranging group of research projects which include development of management strategies, competitive colonization, vaccine development, water cleanup for reuse, irradiation, etc.

Current ARS swine research totals approximately $27.5 M. Like the whole of ARS research, swine research in ARS covers a broad range of topics. Of particular interest to this Symposium is a project on the Identification and Mapping of Genes involved with Parasitic Disease Resistance and Susceptibility and the research to develop controls against parasites, bacterial and viral diseases. Food safety is an important aspect of disease control and ARS research addresses trichinae, toxoplasma, and of course *Salmonella*, the subject of this symposium.
Historical Highlights of Identification and Control

The USDA has had a major role in identifying and controlling Salmonella in food starting with their isolation in 1885 as paratyphoid bacteria or the "hog cholera bacillus" by Salmon and Smith.\(^1\) At that time Dr. Daniel E. Salmon, a veterinarian, was Chief of the Bureau of Animal Industry (BAI) of the USDA. The Bureau had been established by an Act of Congress on May 29, 1884, "to prevent the exportation of diseased cattle and to provide the means for the suppression and extirpation of pleuroneumonia and other contagious diseases of domestic animals." (The BAI was joined with other research units to form ARS during the reorganization of 1953.)\(^2\) Dr. Theobald Smith, the other scientist associated with the isolation of Salmonella was a physician on Dr. Salmon's staff. Thus Salmonella started out as a multidisciplinary concern.\(^3\)

The first identification of Salmonella coincided with the recognition of the need for inspection of meat to protect the public health. In the 1880s, there was domestic press coverage of the lack of hygiene in slaughterhouses, and some European countries restricted imports of American meat. In 1890, the first Federal Meat Inspection Statute was enacted but it applied only to exported meat; amendments were enacted in 1891 and 1895 to provide for inspection of meat for domestic markets, but they were weak and underfunded.\(^4\) In 1906 following the publication of Upton Sinclair's novel, The Jungle, Federal meat inspection authority (P.L. 59-242) was considerably strengthened for all meat moving in interstate commerce, and the 1906 law as modified in 1967 (P.L 90-201) remains the basis for today's meat inspection.\(^5\)

However, the identity of individual strains of the paratyphoid bacteria found by Salmon, and Smith was not clearly established until White in 1926 (Med. Res. Council, Spec. Rpt. Ser. 103, Brit.), recognized the importance of considering bacterial variation in relation to the antigenic analysis of paratyphoid strains. The confirmation and extension of White's work by Kauffmann in 1941 (Die Bakteriologie der Salmonella-gruppe, Ejnor Munksgaard, Copenhagen) resulted in the Kauffmann-White schema for the identification of paratyphoid bacteria. Since Salmon and Smith has isolated and described the first member of the group, the generic name of Salmonella was chosen and Salmonella choleraesuis became the type species.\(^1\)

Salmonella (other than Salmonella typhi) first began to be recognized as a public health problem following World War II. D.H. Udall's The Practice of Veterinary Medicine, Fifth Ed., published by the author in 1947, in a discussion entitled, Paratyphoid in Sheep and Foals, stated that "Reports of (General Bacteriology, 1946), a number of food poisoning outbreaks have been plausibly attributed to S. typhimurium." Alverez (Textbook of Medicine, 1943) writes that the commonest cause of food poisoning due to bacteria or bacterial toxins is contamination of the food with living bacteria belonging to the Salmonella group.

In 1948 Edwards, Brunner, and Moran reported on the occurrence and distribution of the Salmonella types in the U.S. They included samples from humans, animals, water, and food in their study which was published as Kentucky Agricultural Experiment Station Bulletin 525. In 1951 McCullough and Eisele found that Salmonella strains isolated from market samples of spray dried egg powder readily induced salmonellosis in human volunteers. Fifteen years later in 1966 Brunner and Gillespie stated that "From the public health aspect it appears that meat, milk and eggs, products made from these materials, and domestic pets are the most important sources of Salmonella infection."\(^1\)

There was public interest in Salmonella at that time particularly in animal feeds, and in 1959, the USDA undertook a survey and study of the problem, issuing findings in 1961.\(^6\) This was followed by the first National Conference of Salmonellosis held at the National Communicable Disease Center of the U.S. Public Health Service in Atlanta, Georgia in 1964, and publication by the National Academy of Science of "An Evaluation of the Salmonella Problem - A Report to the 
USDA and the FDA" in 1969. However, only further report writing? followed this initial surge of interest; and in fact, pathogen contamination of food was not on the front burner with the general public in this era dominated by chemical residue concern. Salmonella in meat and bone meal in animal feed did receive considerable attention, perhaps from the naive view that elimination of this source of Salmonella would solve the problem. However, Ben Pomeroy did demonstrate that turkeys could be raised free of Salmonella in Minnesota.8

The Food Safety and Inspection Service, particularly through Ralph Johnson, did understand that Salmonella were a public health problem and requested research from ARS in 1980 to help solve the problem, but, except for that relating to S. enteritidis, only post harvest research was conducted at that time. In 1985, ARS realized it was necessary to attack the problem of contaminated meat at the animal production level, and preharvest studies were initiated with broilers by Roy Blankenship at the Richard Russell Research Center at Athens, Georgia. Also in 1985, the Food and Nutrition Board of the National Research Council published, "Meat and Poultry Inspection, the Scientific Basis of the Nation's Program." This was really an epochal volume, but one which did not receive nearly enough publicity at the time.

Three years later, in 1988, the public became acutely aware of Salmonella following the recognition of Salmonella enteritidis infection of eggs. Also in 1988, ARS initiated research to prevent Salmonella in swine with Richard Wood at the National Animal Disease Center. But it was not until 1993, following the deaths of children from E. coli 0157:H7, that the whole food safety issue, including Salmonella, really began to command the nation's attention. Unfortunately, by that time, Congress was in no mood to provide any real increases for ARS or any other agency's pathogen control research programs. However, ARS has been able to redirect in-house resources at 5 locations to address Salmonella concerns in poultry and cattle as well as swine. Of particular success has been the development of a competitive exclusion culture of known identified normal gut flora that is able to exclude Salmonella spp. from the GI tract of newly hatched chicks (see D. Nesbit, Use of Defined Competitive Exclusion Cultures to Enhance Colonization Resistance to Enteric Pathogens, these Proceedings). Initial efforts are now being made to adapt this approach to swine.

REFERENCES

3. Veterinary Medical Science and Human Health, p.28 - Committee on Government Operation, US Senate, August 10, 1961
5. CRS Report for Congress, Meat and Poultry Inspection: Background and Current Issues, 93-574-ENR, June 9, 1993
8. B. Pomeroy, personal communications.
FDA’s Role in Feed Safety

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The Food and Drug Administration (FDA) has primary responsibility in the federal government for food safety in the United States. FDA is charged with the enforcement of the Federal Food, Drug, and Cosmetic Act (FFDCA), and food safety related aspects of the Public Health Service Act (PHSA). The FDA mandate under these Acts includes widespread responsibilities to help ensure preharvest food safety. One mission of FDA’s Center for Veterinary Medicine (CVM) is to regulate the levels of contaminants permitted in animal feeds to ensure that the food for man and animals is safe and free of illegal drugs, industrial chemical, pesticide residues, and harmful bacteria. To meet this mandate, CVM has emphasized the application of HACCP programs in the feed industry as an approach for achieving salmonella negative feed. To establish a baseline in the feed industry, CVM has conducted surveys to determine the prevalence of salmonella contamination in animal and vegetable proteins, complete feed, and the primary protein meal ingredient.

Complete feed and primary meal ingredient samples were collected from commercial feed mills and on-farm mixers. The on-farm mixers were randomly selected from FDA’s Official Establishment Inventory (OEI). Each district was assigned a specific number of commercial feed mills based on the number of registered feed mills within the district. The district was instructed to select mills that would be representative of the feeds manufactured in the district. Medicated feeds were included in the sampling. Each sample of meal and complete feed consisted of 30 individual sub-samples which were aseptically collected. Analysis followed the procedures outlined in FDA’s BAM and included serogrouping and serotyping. The results being reported today represent data from 1,980 meals subsamples and 1,860 complete feed subsamples covering 66 meals and 62 complete feeds respectively.

Sixteen percent of the complete feeds and 48% of the meals were positive for Salmonella. When the meals were grouped by animal and vegetable source, 82% of the animal meals and 37% of the vegetable meals were positive for Salmonella. When the meal and complete feed pairs were compared, if the meal was positive, the complete feed was also positive in 30% of the samples. There was one instance in which the meal was negative and the complete feed positive (3%) and 32 instances in which both the meal and feed were negative (97%).

A more complete text can be located after the breakout group reports.

The following is a summary of the research conducted by Dr. Amy Waldroup and her co-workers at the University of Arkansas under an FDA contract. The research was to optimized the culture conditions for detecting Salmonella in a feed matrix and to evaluate several rapid detection kits for their ability to detect Salmonella in a feed matrix. The rapid detection kits were used in accordance with the manufacturers directions. This summary incorporates Dr. Waldroup’s comments and was prepared by Dr. Dan McChesney of FDA / CVM.

SUMMARY

Results obtained from these studies suggest that the culture method described by Bailey and Cox (1992) for the simultaneous recovery of Salmonella and Listeria from food samples can also be used for detection of Salmonella in feed ingredients and finished feeds. This culture method, which includes preenrichment in Universal Preenrichment broth, is capable of detecting as few as 1 to 2 salmonellae
(either nalidixic acid-resistant or indigenous species) in 100 g of finished feed or animal byproduct meal. It should be noted that in all of the present studies sample size was 100 g due to the extremely low level of salmonellae typically reported for feeds and feed ingredients. Universal preenrichment broth was superior to lactose broth for the detection of indigenous salmonellae in meat and bone meal, regardless of assay procedure.

In an interlaboratory study involving five laboratories, the Bailey and Cox (1992) culture procedure resulted in 1.3% false negative feed or feed ingredient samples and no false positive samples. The SAL-TEK procedure resulted in 3.1% false negative samples, and 6.2% false positive samples. However, half of the false positive samples and 3 of 7 of the false negative samples which occurred when the SAL-TEK procedure was utilized were from a single laboratory. Thus, if only the data from the other four laboratories is considered, the culture method of Bailey and Cox (1992) and the SAL-TEK procedure have almost identical false negative and false positive frequency rates.

Based on these studies, the contractor suggested a minimum sample size for finished feeds or feed ingredients of 100 g, preenrichment in Universal Preenrichment broth, followed by either a standard culture procedure or the SAL-TEK immunoassay.

In addition to the SAL-TEK, the GENE-TRAK, CAP-TEK, TECRA UNIQUE, and direct plating of Salmonella on immunobeads were evaluated for the ability to detect Salmonella in naturally contaminated feed samples.

The GENE-TRAK procedure resulted in no false positive samples, but did result in 10.3% false negative samples. These false negative samples were spread out among various inoculum levels and even included samples to which salmonellae were added at the rate of 140 CFU/100 g feed.

CAP-TEK procedure resulted in a higher number of false positive and false negative results than with SAL-TEK or the culture method. For samples with very low levels of, the elimination of the selective enrichment step (as in the CAP-TEK method) may not be advisable.

The TECRA UNIQUE method produced an unacceptable number of false negative samples. The number of Salmonella present in the preenrichment culture was postulated by the author to not to have been high enough for detection by the TECRA UNIQUE method. Earlier preliminary tests utilizing TECRA UNIQUE for feeds with known levels of inoculated Salmonella had shown it to be comparable to the culture method.

The direct plating of Salmonella on immunobeads also produced too many false negative results. A potential problem noted with this method was that it was extremely labor-intensive. This method did, however, reduce detection time and media needed for samples that contain moderate levels of Salmonella.

In summary, a series of studies utilizing naturally contaminated feed ingredients or finished feeds obtained from FDA, suggested that for feed ingredients or finished feeds either the SAL-TEK or the culture method of Bailey and Cox (1992) will provide the most accurate and consistent results. Because of the low levels of Salmonella in the naturally contaminated samples tested, extreme reductions in the total time allowed for growth may not be advisable and could lead to an unacceptable number of false negative samples.

Finally, it was noted by Dr. Waldroup that it would be very difficult to perform either the culture method or the SAL-TEK at a facility that did not have any standard microbiological equipment. The typical feed mill will probably not have the required facilities or equipment for either the culture method or a rapid kit.
Culture Method Utilizing Universal Preenrichment Broth (UPB)
Bailey and Cox, 1992

1. Add 100 g of feed sample to 900 ml of UPB by sprinkling feed onto the surface of UPB. Allow the feed to soak undisturbed for 30 minutes. Mix well, loosen caps if bottle is used and incubate for 18 to 20 hours at 35 C. Because of the large quantity of UPB it is advisable to prewarm the liquid to 30 to 35 C prior to adding sample. Alternately, large freezer ziploc bags can be used to hold sample and UPB if these bags can be held upright in incubator.

2. Swirl the sample gently, allow loose particles to settle and transfer 1.0 ml of incubated broth to tubes of tetrathionate-Hajna broth (10 ml/tube) and 1.0 ml to selenite cystine (10 ml/tube). Incubate tetrathionate-Hajna tube at 42 C for 20 to 24 hours. Incubate selenite cystine tube at 35 C for 20 to 24 hours.

3. Streak incubated tetrathionate-Hajna and selenite cystine broths onto separate brilliant green sulfa, modified lysine iron agar, and XLT-4 agar plates. It is convient to use tri-plates for these three selective agars. Incubate plates for 24 to 26 hours at 35 C.

4. Pick, stab, and streak typical colonies onto triple sugar iron and lysine iron agar slants. Loosen caps and incubate for 24 hours at 35 C.

5. Serologically screen with poly O somatic antigen using the slide technique.

6. For atypical colonies, biochemically characterize isolates with conventional methods or API or Micro-ID miniaturized kits.

7. Serologically confirm using poly H flagellar antigens. Use the tube technique.
FSIS Overview

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The FSIS Animal Production Food Safety Program (APFSP) staff is listening to its stakeholders by conducting commodity needs assessments, establishing and maintaining contacts with the commodities at the policy and technical level, and organizing meetings such as the National Forum held in May of 1995.

The APHIS Veterinary Services Food Safety Management Team initiated formation of several focus groups in 1993 with representatives from the ARS, APHIS, CDCP, FDA, ERS, and FSIS to provide direction for preharvest research activities. One focus group concentrated on Salmonella. It determined that it was important to do prevalence studies to answer such questions as what percentage of animals are carriers, what species serve as reservoirs, if there is a higher level at slaughter as opposed to on farm, and what role transportation and marketing play. The focus group also stressed that monitoring and surveillance is an important, ongoing process by which we detect trends or changes. The focus group recommended improved diagnostics and the use of experimental modeling to help predict the effectiveness of proposed interventions.

Currently, APHIS and FSIS are analyzing data from the Swine 95 NAHMS project. Preliminary findings will be presented by an APHIS epidemiologist at this meeting. Some highlights of the study include the finding that E. coli 0157:H7 has not been isolated from market swine. Yersinia, which researchers think may be the next emerging zoonotic pathogen in swine, was found in 13.3% of samples. Campylobacter which causes a significant amount of human illness was found in 24% of the samples. These results add to our knowledge of baseline prevalence and help determine possible managerial risk factors.

For APFSP to make recommendations for present and future policies based on science, epidemiological studies are the first step. Comparable baseline prevalence levels must be developed, followed by risk factor analysis through follow-up studies. Intervention strategy evaluation and cost benefit analysis help determine if we are going in the right direction.

The FSIS APFSP's goals are to enhance private investment in needed applied studies and to provide opportunities for private/public partnerships where funding is available. We envision a review board process for applied studies to assist FSIS in achieving these goals. The process begins with a standing board made up of representatives from federal agencies (FSIS, APHIS, ARS, CDCP, FDA, CSREES, and ERS). The standing board will be charged with developing a process for needs assessment and prioritization. If APFS receives funds for focused applied projects, this board will call for proposals, evaluate them, appropriate funds and develop an accountability process. There will be a technical expertise group with members representing government, industry, academia, and the private sector. This group will be called upon to provide answers on scientific aspects of animal production food safety. A stakeholders group (consumers, commodity representatives, producers, etc.) will provide overall vision of their concerns, feedback to stakeholder groups they represent, collaboration opportunities, avenues to leverage resources, and "real life situation" expertise in the design, development and implementation of applied field research initiatives.
Incidence and Control of Salmonellosis in Swine: 
The Public Health Relevance

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The incidence of clinical salmonellosis in swine remains a difficult, if not an impossible assessment. The variables of husbandry, stress, sampling methodology, serotypes isolated, sanitation/hygiene, climate, all contribute to the complexity of the Salmonella problem, not only in swine, but other livestock and poultry. A large percentage of swine are asymptomatic carriers of Salmonella, creating a serious challenge to control initiatives. Equally relevant is the well accepted theory that shipping and other stress related activities increase the number of salmonellae shed in the feces. This could possibly heighten the potential for the organism to enter the food chain and obvious food safety concern.

The four main isolates of swine in the United States continue to be S. choleraesuis, the predominant serotype, about 65% of the isolates; S. derby, 8%; S. typhimurium, 6%; and S. agona, 3-4%. One of the very interesting aspects of the entire control/prevention perspective is that although S. choleraesuis is doubtless the most common isolate of swine, human infection with S. choleraesuis is rare. What inference, if any can be made? And, indeed, in humans, S. choleraesuis will not be overlooked because this serotype is especially pathogenic with a high case of fatality rate.

Salmonellosis in swine will continue to be a significant problem because of the existing variables. Sanitation/hygiene and husbandry will remain a resource for control. The production of an effective vaccine will prove to be a positive impact both at the farm level and the public health relevance.

The rendering industry through the Animal Protein Producers Industry (APPI) Salmonella reduction/education program and its planned objective to implement Hazard Analysis and Critical Control Points (HACCP) throughout the industry to assist in the production of a safe feed should add impetus to the prevention/control initiative.
NPPC Perspective on Food Safety

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U. S. consumers expect to be able to buy safe, high quality food at a reasonable cost. Consumer confidence in the safety and wholesomeness of pork products is critical to reaching the industry's goal of being "The Meat of Choice™."

National Pork Producers Council's Food Safety Activities

NPPC formed a Pork Safety Task Force in 1994 with the mission of assuring the safety of U.S. pork through coordinated, science-based efforts throughout the pork chain. The long term goals of this Task Force are to decrease the potential for foodborne illness from pork products and to improve product image with regard to safety among consumers world-wide. The Pork Safety Task Force recognized the need for more on-farm food safety research to determine the points in the food chain where interventions will result in enhanced food safety.

Areas identified as needing research included:

- Identification and estimation of the potential impact of microbiological and parasitic on-farm hazards that can pose risks to public health
- Identification of critical control points to prevent, eliminate, control, or reduce on-farm hazards under various pig management systems
- Economics of implementation of HACCP models
- Investigation of the effect of pig handling prior to and during transportation on the potential for transmission of pathogens and carcass contamination
- Effect of feed withdrawal prior to slaughter on fecal contamination of swine carcasses
- Identification of the relationship between prevalence of potential pathogens in swine herds, in swine during and after transport, and on swine carcasses

In 1994, NPPC with several state associations funded nine animal production food safety projects. In 1995, NPPC funded six projects. The majority of the funded projects included Salmonella as the focus.

We hope one of the outcomes of this symposium will be to develop a more definitive research agenda for Salmonella and to facilitate the coordination of research efforts of the private and public sectors.
CSREES Funding for *Salmonella* in Swine and Pork Production Research

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During fiscal years 1992-1994, the Cooperative State Research, Education and Extension Service (formerly the Cooperative State Research Service and the Extension Service) funded 11 projects related to *Salmonella* in pork production. Most projects were funded in the Northcentral Region (IL, IA, KS, MO, NE) and were multi-year awards. In addition to Hatch Act Regional Research funding, awards were made as part of the National Research Initiative Competitive Grants Program, the Animal Health and Disease Formula Program, and the Special Research Grants in Animal Health.

As future research priorities are determined, CSREES will seek to leverage research dollars through collaboration and broadened participation of our partners in all program areas. Enhanced access to research information will be a part of the commitment of CSREES to facilitating the work of the 21st century researcher.

**ADDENDUM**

**Selected Internet-based Resources**

**HACCP**

* The International Meat and Poultry HACCP Alliance was developed on March 25, 1994. It is within the Institute of Food Science and Engineering's (IFSE) Center for Food Safety. The International Meat and Poultry HACCP Alliance was developed to provide a uniform program to assure safer meat and poultry products.

http://ifse.tamu.edu/haccpall.html

* FoodNet, an information network for the food industry, is a joint pilot project of the Food Institute of Canada and Agriculture and Agri-Food Canada.

http://foodnet.fic.ca/welcome.html

*(HACCP Symposium - November 12, 1995)*

http://www.cvm.uiuc.edu/haccp/symposium/title.htm

**Food Safety**

* The National Food Safety Database (NFSD) has been produced to educate food handlers about ways to prevent foodborne illness. This Database contains comprehensive food safety information authored by numerous academic, government and private organizations.
http://www.agen.ufl.edu/~foodsafe/foodsafe.html

* Food Safety Consortium dedicated to the enhancement of food safety through research on pork, beef, and poultry products.

http://www.public.iastate.edu/~foodsafety_info/homepage.html

* Food and Nutrition Information Center (NAL)

The USDA/FDA Foodborne Illness Education Information Center provides information about foodborne illness prevention to educators, trainers, and organizations developing education and training materials for food workers and consumers.

http://www.nal.usda.gov/fnic/foodborne/foodborn.htm

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**Funding Sources and Research Information**

* Community of Science access to Federally-Funded Research in the U.S.

http://cos.gdb.org/best/fed-fund.html

* Current Research Information System


* The National Research Initiative Competitive Grants Program (NRICGP) is charged with funding research on key problems of national and regional importance in biological, environmental, physical, and social sciences relevant to agriculture, food, and the environment on a peer-reviewed, competitive basis. The NRICGP supports a spectrum of research ranging from basic, fundamental questions relevant to agriculture in the broad sense to research that bridges the basic and applied sciences and results in practical outcomes. Competition is open to scientists at all academic institutions, Federal research agencies, private and industrial organizations, and those individuals qualified but not affiliated with one of the aforementioned organizations.

http://www.reeusda.gov/new/nri/nricgp.htm

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**ISO 9000**

http://www.iso.ch/9000e/forum.html

The NAHMS Swine '95 Baseline study involved two telephone interviews by NASS in June and December 1995. Farms from the top 16 swine states with a specified number of grower/finisher animals were eligible for participation in the NAHMS Swine '95 Grower/Finisher study. Two on-farm visits were made by APHIS:VS Veterinary Medical Officers (VMO's). The first visit was completed between July 17 and September 15, 1995. The second visit was completed between November 6, 1995 and January 19, 1996. In addition to completing two questionnaires, VMO's collected samples of blood, feces, and/or feed.

Up to 30 blood samples were taken per farm, no more than 15 samples coming from breeding animals, the remainder being collected from late finishers (within 30 days of being marketed). Up to 50 fecal samples were taken (from pens containing late finishers) on 160 farms resulting in the collection of 8000 fecal samples to be evaluated for Salmonella. Fecal samples were to be collected from no more than 10 pens. Five 100 g feed samples were taken from the ration to late finishers to be tested for Salmonella.

Fecal samples will be used to identify the presence of Salmonella in herds. Feed samples will be used to identify the presence of Salmonella in swine feed. Researchers at the NADC and NVSL will identify the group, serotype, and antimicrobial resistance patterns for Salmonella isolates.

The NAHMS Swine '95 study will help identify on-farm factors that may be useful in controlling the shedding of these organisms and reduce the risk of food borne illness due to the consumption of pork. It will also evaluate the associations between detection of Salmonella by blood, feces, and feed.

In addition to the above, fecal samples from the pilot study were used by Dr. Gbadamosi at Tuskegee for research on the development of PCR for Salmonella.
An On-Farm Survey of Swine Feeds and Feed Ingredients for *Salmonella*, and Identification of Associated Herd Risk Factors

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Research during the last year has centered on conducting a survey for the occurrence of *Salmonella* spp. in the feed and feed environment on the pig farm and identifying risk factors which may be related to such occurrence.

METHODS

Samples of feed and feed ingredients were collected from 30 pig farms in eight states and cultured for the presence of *Salmonella* spp. Information was collected on physical and managerial characteristics of each farm for risk factor analysis.

RESULTS

Salmonellae were isolated from at least one feed or ingredient in 14 (47\%) of the 30 herds surveyed, representing five states. Of a total of 1264 samples, salmonellae were recovered from 36 (2.9\%). Thirteen different serotypes and two that were untypable were found. The isolation of *Salmonella* spp. in the feed had a statistically significant association with six of the herd characteristics surveyed, including the lack of bird-proofing measures employed on the farm (p=0.03), using finisher feed that was prepared on the farm versus purchasing such feed (p=0.008), and housing pigs in facilities other than total confinement for the growing (p<0.025), finishing (p<0.025), gestation (OR=27, 95\%CI:1.305-555.57), and breeding (p<0.005) stages of production.

CONCLUSIONS

Salmonellae were relatively easy to isolate in the farm feed environment when even a relatively small sample size, compared to the overall volume of feeds and ingredients on the farm, was taken. Certain management practices may be related to the occurrence of *Salmonella* spp. in the farm feed environment.
Microbiology of Pig Carcasses

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A study focusing on the microbiology of carcasses from pigs with differing origins and feed withdrawal times was conducted. Four buying sources (terminal market, buying station, outdoor farm, and confinement farm), four feed withdrawal times (0, 2, 4, and 6 h) and the incidence of puncture GI tracts during evisceration was studied to determine the incidence of pathogens (including \textit{Salmonella} spp.) as well as spoilage organisms on the carcasses. Of the 932 samples tested, a 0\% incidence of \textit{Salmonella} spp. occurred.

A recent two-part study was designed to determine the effects of trim (hot-fat trim - HFT or non-fat trim - NFT) and chill (normal chill - NC or freeze chill - FC) methods on the microbial load of pork carcasses. Composite ham, loin, belly, and shoulder samples produced only one \textit{Salmonella} spp. positive from sixty samples. No positive samples were found among the 14 d aged hams and loins from the same carcasses.

In these two studies, the incidence of \textit{Salmonella} spp. was very low, but was detected on one sample.
Research on Persistent Colonization of Pigs by *Salmonella typhimurium* and the Effects of Transportation Related Stress on the Shedding of *Salmonella typhimurium*

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Research in my laboratory has been on the mechanism(s) employed by *Salmonella typhimurium* to persistently colonize pigs and on the factors contributing to increased shedding of *S. typhimurium* by pigs at slaughter. A phenotype of *S. typhimurium* has been identified that attaches to epithelial cells isolated from the pig small intestine. Cells of the adhesive phenotype produce pili that may be the adhesin, while cells of the non-adhesive phenotype do not. Cells of the adhesive phenotype also produce 10-12 unique envelope proteins and several new surface antigens. Adhesive cells are more readily phagocytized by porcine neutrophils and macrophages and have a much greater degree of intracellular survival in the phagocytic cells. Cells can readily shift between the two phenotypes. In the laboratory the rate of change is between $10^{-2}$ and $10^{-4}$. When pigs were challenged with cells in the non-adhesive phenotype, only cells in the adhesive phenotype were recovered from pigs. Both phenotypes were of equal virulence. This demonstrates that the adhesive phenotype is important in pigs. A non-adhesive mutant was isolated and shown to be less virulent in mice and was more rapidly cleared from the intestinal tract of pigs. The role of the adhesin and the other properties associated with the adhesive phenotype are being investigated with the intent of learning how pigs can be long term carriers of *S. typhimurium*.

The stress associated with shipping has been associated with increased shedding of *Salmonella* from pigs. The result of this is to increase the risk of spreading this food borne pathogen at the slaughter plant. However, since most of the evidence for this perceived association between stress and increased shedding of *Salmonella* is anecdotal, we have initiated a systematic approach of this problem. Pigs infected with *S. typhimurium* will be subjected to transportation related stress and quantitative measurements of *Salmonella* shedding from each pig measured to determine if shipping stress does increase shedding. To ensure that all pigs in the study were carriers of *Salmonella*, 30 pigs at 4 weeks of age were challenged with a strain of *S. typhimurium* known to persistently colonize pigs and that can be easily cultured from fecal samples because it is resistant to nalidixic acid. These pigs will be reared in isolation until market weight. One-half of the pigs will be transported by truck for 3-4 hours, brought to the meat processing plant at the University of Illinois, and held in pens for 1-2 hours prior to slaughter. The other pigs will be slaughtered without the long transport and holding. Each pig will be sampled for fecal *Salmonella* prior to transport and again after slaughter. Immunologic variations among groups also will be measured including antigen-specific T-cell responses, measurement of IFNγ producing cells, and measurement of other cytokines. This study will allow us to confirm the relationship between transportation induced stress and shedding of *S. typhimurium* and to begin to define the mechanisms that increase shedding of *Salmonella*.
The Effects of Feed Deprivation on Shedding of *Salmonella typhimurium* in Swine

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The role that management decisions on swine production units play in both food safety and the on-farm ecology of human and animal pathogens such as *Salmonella* spp. has yet to be defined. Two management areas that may have profound impact on *Salmonella* ecology are transport and feed deprivation. Both transport and feed deprivation are animal stressors and may predispose asymptomatic carriers of *Salmonella* to shed. A pattern of shedding in response to stress has been shown to occur in cattle and poultry. The following information reflects results from a study of market hogs. Pigs from this production unit were known to be asymptptomatically infected with *Salmonella typhimurium*.

**Study Design and Results:** Study pigs (n=118) from a commercial finishing floor (all in-all out) were randomly allocated to one of two groups: a full feed group prior to slaughter and 24 hour feed deprivation prior to slaughter. Fecal samples were collected from all pigs 48 hours prior to slaughter and evaluated for the presence of *S. typhimurium*. Twenty four hours prior to slaughter, pigs were weighed and feed removed from pens of the feed deprived group. Six hours prior to slaughter, pigs were loaded onto a truck and transported to slaughter, transport time was three hours. At slaughter, intestines were removed and weighed and rectal contents and ileo-cecal regions were sampled and evaluated for the presence of *S. typhimurium*.

Feed deprived pigs had significantly reduced gut fill at slaughter. Prior to shipping, approximately 12% of pigs from both groups were found to be shedding *Salmonella* spp. Following shipping, there was a small increase in the rate of *Salmonella* shedding from both groups. The full feed pigs were consistent in shedding pre- and post shipping while the feed deprived group had more pigs change *Salmonella* status pre- and post-shipping.

**Future Directions:** Other results of the study suggested that feed withdrawal affected carcass quality. A concern regarding the results was that the hot and humid weather during the study period might have confounded the results, particularly since a previous study on feed withholding suggested that it did not effect carcass quality. We plan to repeat the study in cool weather and add an additional group with a shorter feed withhold (18 hours).
Salmonella in Swine Research Projects in Denmark and EU

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Salmonella spp. in pork has become recognized as a major source for human salmonellosis in Denmark. Consequently, a number of research activities, surveillance and control programs have been initiated during the last 4 years.

Feedmills and feedstuff

Feedmills were found to be contaminated with many serotypes and feedstuff was recognized as a source of Salmonella spp. in swine herds. Today, all feedmills follow a mandatory Salmonella spp. control program. In 1995, the level of Salmonella spp. in raw materials and product were 5% and 0-1%, respectively.

Diagnostic assays

A new test for serological detection of specific Salmonella antibodies in swine serum and meat juice was developed, and is now used for routine surveillance of all breeding, multiplying and slaughter herds in Denmark.

Epidemiological typing methods

Pheno and genotyping methods for routine surveillance and outbreak investigations were implemented or developed: serotyping in microtiter plates, phage typing, antibiogram typing, plasmid profiling, ribotyping, and pulse field gel electrophoresis.

National project: Reduction and control of Salmonella in pig herds

A 3 year research project was launched in January 1994 as a joint venture between the Danish Ministry of Agriculture and Fisheries and the Federation of Danish Pig Producers and Slaughterhouses. The project should provide the scientific basis for the recommendations given to pig producers and consists of 7 parts: 1) HACCP in pig herds, 2) herd management strategies, 3) eradication strategies, 4) vaccination, 5) immunological background for the intracellular carrier state, 6) epidemiological investigations, 7) database-epidemiology.

EU project: Salmonella in pork (SALINPORK)

A 3 year research project beginning in the spring 1996. The participating countries are with number of scientists in brackets: Denmark as coordinator (17), The Netherlands (13), UK (5), Greece (5), Sweden (5), and Germany (2). The over-all objective is to establish the epidemiological basis, to develop the diagnostic tools, and evaluate options for control of Salmonella in pork at the pre-harvest and at the harvest levels.
Investigation of *Salmonella* Contamination of Pigs in Australia

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Microbiological surveys of pigs before, during and after slaughter and processing have been conducted at abattoirs in Australia, to estimate the incidence and risk factors for *Salmonella* contamination on carcasses. In one such survey, approximately 10% of carcasses were positive for *Salmonella* contamination at the end of the slaughter line, although there was considerable variation between abattoirs and between herds in the incidence of contamination. A range of *Salmonella* serotypes was recovered from carcasses, with the most common being *S. derby*, *S. give*, *S. anatum*, and *S. ohio*. The incidence of *Salmonella* contamination on carcasses varied along the slaughter line: some contamination on the skin surface of carcasses survived scalding and dehairing, despite the temperatures achieved during these processes.

Analysis of risk factors associated with *Salmonella* contamination on carcass surfaces following slaughter indicated that the source of pigs, transport time and distance, lairage time, and intestinal carriage of *Salmonella* influenced the incidence of carcass contamination.

As an extension to the survey of *Salmonella* contamination on carcass pigs, the pig industry in Australia has commissioned a nationwide program to enhance the microbiological quality of pig meat. One of the aims of the Pig Meat Hygiene Program is to determine baseline levels for contamination of carcasses and fresh meat, at abattoirs and retail outlets in the major metropolitan centres in Australia, with respect to the following organisms: quantitative assessment for total viable counts (at 50°C and 250°C), total coliforms, *Campylobacter jejuni/coli*, *Pseudomonas* spp (fresh meat only), and qualitative assessment for *Salmonella* spp, coagulate positive *Staphylococcus aureus*, *Yersinia* spp, *Listeria monocytogenes*, and *verotoxigenic/enterohaemorrhagic E. coli*. Training programs have been developed, and are being conducted throughout Australia, for meat processors and QA managers to improve processing techniques that influence microbiological quality. In addition, it is proposed that a *Salmonella* ELISA will be investigated under Australian conditions, to determine its value as a tool for reducing the entry of *Salmonella* into abattoirs by way of intestinal infection in pigs, thereby reducing the level of carcass contamination following slaughter.

Also as part of the Pig Meat Hygiene Program, HACCP protocols are being developed for the production and processing of pigs, by means of a collaborative process involving researchers, industry and regulatory agencies. The aim of this approach is to develop effective and practical methods for assuring, and enhancing, the microbiological quality of carcass and pig meat throughout the industry in Australia.
Salmonella Reduction at the Farm Level

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Introduction

Study A. Three experiments were performed to study the influence of removal of pigs at different ages prior to the age when *S. typhimurium* had been detected in subclinically infected herds.

Study B. Based on these results, a model for Salmonella reduction on herd level was established. The model consisted of a microbiological survey in the herd to locate infected parts of the herd. Based on the microbiological results a plan for reduction of *Salmonella* was described for each individual farm. A typical plan consisted of hygienic measures combined with all in-all out measures on either pen-level or section level, in combination with an attempt to improve what we define as "enteric health" (absence of diarrhoea) in finishers without use of antibiotics.

Study C. A cross-sectional study was performed using data from a data-base from a Danish slaughterhouse cooperative.

Material and methods

Study A. Experimental design: Three herds (A, B and C) with persistent high levels of infection with *S. typhimurium* were selected. Serological examinations performed for at least 3 months prior to the time of the investigations showed a high seroprevalence (above 50 %) of *Salmonella* infection in finishing pigs in all three herds. All herds used heat treated compound feed. No medical routine treatment was used before or after removal of pigs. 46 pigs were removed at weaning from herd A, 700 pigs were removed from the nurseries in herd B and 98 pigs were removed from the growers unit in herd C. Blood samples, pen samples and/or cecal samples were collected at slaughter.

Study B. Criteria for success: Samples obtained after intervention should place the herd in infection level 1.

Definitions: All in-all out on pen or section level means, that pigs are not moved between pens or sections. Pens or sections are cleaned between batches, precautions are taken to avoid transfer of infected feces between pens or sections.

Procedure: Management of nurseries were changed to all in-all out. Growers sections were either dropped, changed into all in-all out or emptied and cleaned once, and then used as all in-all out on pen level. In finishers sections all in-all out was performed either at pen level or section level. Hygienic procedures were improved, and transport of fecal material between sections or pens were minimized. Organic acids were administered in either water, fermented wet feed or dry feed in 6 herds.

Table 1. Effect of different types of intervention

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In each herd approximately 30 blood samples were collected 5-6 times over a period of 3 months before herd intervention. For a period of at least 3 months after intervention, approximately 30 blood samples were collected 6 times. Only pigs ready for slaughter were sampled to obtain samples from pigs of comparable age.

Study C. A database with information from 5000 herds was used. All herds with insufficient or inconsistent data were rejected. 1531 herds were used for the analysis. Cases were defined as
herds with more than 33 % seropositive meat-juice samples in 1995. Non-cases were herds with less than 33 % seropositive meat-juice samples in 1995. Statistical analysis was performed using SAS genmod procedure.

Results

Study A. No Salmonella was isolated from removed pigs in the three experiments. All blood samples were seronegative. S. typhimurium was isolated from controls in herd A and B (Controls in herd C were not examined bacteriologically). In herd A 39 out of 40 controls seroconverted, in herd B 10 out of 88 controls seroconverted and in herd C 16 out of 30 controls seroconverted.

Study B. Table 1 shows results of intervention.

Study C. The two factors with the greatest impact on the Salmonella level was use of home mixed feed (OR=0.17 Confidence interval 0.08-0.36) and use of fermented wet feed (OR=0.10 Confidence interval 0.02-0.45).

Discussion

Study A. In three herds it was proven feasible to raise finishers void of S. typhimurium infection by removal to clean and disinfected facilities, despite the fact, that the pigs were born in herds with a high level of Salmonella infection in finishing pigs. It seems reasonable to assume, that the control pigs raised within the infected units were infected from either residual infection in the pens or from older pigs in the unit.

Study B. It is difficult to obtain results similar to results obtained by removing pigs to external facilities outside infected herds. No herds reached a zero level. Even when all in-all out management is performed, some herds are not able to reduce the seroprevalence sufficiently. The beneficial effects of fermented wet feed could in part be due to organic acids produced by fermentation (see study C). In all herds where organic acids were part of the intervention, a sufficient reduction was achieved.

Study C. S. typhimurium is the most important serotype in Danish swine herds. This serotype is not isolated from Danish feedstuff. The effects of wet feed and home-mixed feed is not a result of Salmonella in the feed, but probably an effect on the pigs resistance to Salmonella.
Survveillance and Control Program for *Salmonella* in Swine: The Danish Action Plan

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The Danish national *Salmonella* surveillance and control program in swine, which has existed in its present form since January 1, 1995, is mandated by the Act of Zoonoses of December 21, 1994, and implements Paragraph 1, Article 8, of the Zoonosis Directive 92/117/EEC. The objective of the program is to reduce the prevalence of *Salmonella* in swine herds as well as in pork, and represents a comprehensive effort by the swine industry and the veterinary authorities. Results from the first year the program has been in effect were reported by Emborg et al. (1).

The Danish *Salmonella* action plan consists of three parts: (1) Monitoring of (i) herds producing more than 100 finishing pigs a year and (ii) breeding and multiplying herds; (2) Monitoring of animal feeds. In 1993 regulations were passed to make it mandatory for feed mills to submit a *Salmonella* monitoring scheme, including analysis of ready-mixed feed stuffs as well as process control (3). Monitoring of slaughteringhouses including (i) slaughter carried out using special hygienic precautions, (ii) visible fecal contamination and (iii) meat end product. The herd monitoring scheme was described by Bager et al. (2). The serological technique used was developed by the Danish Veterinary Laboratory for use on serum and has been modified to be used on meat juice from the slaughtered pigs (3). (1.i) The surveillance program constitute representative sampling of 18,500 herds from which 850,000 samples are examined every year. On the basis of the proportion of samples reacting positively a herd is assigned to one of three levels. Level 1 with no or very few reactors and no intervention in the herd required. Level 2 herds contain a proportion of reactors and the owner is required to seek advice of how best to reduce the *Salmonella* prevalence in the herd. Level 3 herds contain a high proportion of reactors. As in Level 2, the owner is required to seek advice, and pigs from that herd have to be slaughtered using special precautions to avoid contamination of meat, to minimize the risk of salmonella contamination. (1.ii) The voluntary *Salmonella* surveillance program for breeding and multiplier herds requires that a random sample of 20 blood samples be collected once a month and tested for the presence of salmonella antibodies (3.i). Contamination of the carcass occurs principally when the gastrointestinal tract is removed (1). A plastic bag is everted over the anus and posterior rectum, after it has been cut loose by a rotating knife, thereby minimizing contamination. In addition pigs are not to be fed within 12 hours of slaughter so as to reduce contamination of carcasses by stomach contents. Swine delivered from Level 3 herds have to be slaughtered as late in the day as possible. The heads of the carcasses are not split during dressing, and the thoracic and abdominal viscera are either rejected or heat treated. The carcasses are also tested at random microbiologically by swabbing 1400 cm² area of the skin to determine if *Salmonellas* have been transferred to the carcass during dressing. If more than 25% are positive, all subgroups of pigs from that day’s special precautionary slaughter will be subjected to heat treatment. (3.ii) All slaughter plants are required to monitor fecal contamination of carcasses twice a day on a random basis. The results are reported to the Danish Veterinary Directorate’s Department of Meat Control. (3.iii) Monitoring of pig meat end products happens by rolling surveillance of about 2200 random samples every month, and is an indication of the effectiveness of the Danish *Salmonella* surveillance program. The number of samples collected is determined by the number of pigs slaughtered in that plant.
References
The Swedish Salmonella Control Program with Special Reference to Pig Meat Production

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In 1953-1954 a severe Salmonella epidemic, involving almost 9000 people, occurred in Sweden (1). This event clearly demonstrated the need for a Salmonella control program. In 1961 the first governmental regulation concerning Salmonella control was implemented. A comprehensive description of the control program was presented at the International Course on Salmonella Control in Animal Production and Products (2). After joining the EEC some parts of the program were revised (3).

The Salmonella control is regulated by parliamentary law and covers animals, feed, food, human health and import restrictions. It regulates the power of the authorities, the routines in case of Salmonella infections in domestic animals, the obligations of the animal owner and the financial support to animal owners in cases of Salmonella infections in domestic animals. It also regulates domestic feed production and gives rules and recommendations regarding production, hygiene and monitoring of Salmonella. The import of certain animals and animal products is also regulated.

All isolations of Salmonella are notifiable. All isolates of Salmonella are characterized by serotyping the strains.

The Salmonella control program in swine has the following cornerstones:
1. Control of imported feed raw materials and domestically produced feed (6).
2. Testing for Salmonella of all sanitary slaughtered animals as well as any suspect animal at normal slaughter (3).
3. Surveillance at all slaughterhouses, including testing of individual animals (intestinal lymph nodes) as well as hygiene control (swab samples) (3).
4. Surveillance of breeding herds at farm level (fecal samples) (3).
5. General surveillance by clinical checks made by practicing and animal health veterinarians.
6. If Salmonella is found, infected herds are put under restrictions until the herd is considered free from infection. Farmers will be partly economically compensated for costs due to the restrictions.

The reported number of infected pig herds has continuously decreased. In the 1970’s approximately 35-40 infected pig herds were reported annually. After 1979, on average, 3-4 infected pig herds have been reported annually (4,5). Since 1986 most outbreaks have occurred in fattening herds and have been caused by Salmonella derby. In a few herds infections persisted but measures taken seem to have eliminated the infection.

Surveillance during 1994-1995 showed a very low level of Salmonella infected/contaminated pigs. Only 15 (0.2%) out of 8888 examined slaughtered pigs were infected with Salmonella, 3 (0.03%) out of 8876 examined carcasses were contaminated with Salmonella and no Salmonella was found in fecal samples from approximately 8500 pigs originating mainly from elite breeding herds.

3. Swedish Salmonella Control Programmes for Live Animals, Eggs and Meat, Commission decision of 23
Feb. 1995
5. The Swedish Board of Agriculture, Records of outbreaks of *Salmonella*
Pathogenesis, Transmission, and Control of Salmonellosis in Swine

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The objectives of our project at the National Animal Disease Center are:

1. To identify virulence factors important in the pathogenesis of *Salmonella choleraesuis* and *Salmonella typhimurium* in swine
2. To define the epidemiology and transmission of *Salmonella* in swine
3. To define the porcine immune response to acute and chronic *Salmonella* infection focusing on mechanisms to reduce or eliminate the pathogenic organism
4. To identify methods to control *Salmonella* in swine

Progress for these objectives has resulted in the following information:

1. We produced soluble antigen extracts for the development of indirect ELISA assay which may be used to monitor the immune response and identify carrier animals. A mixed ELISA format, as described by the Danish researchers and designated SalAD, was developed. The sensitivity and specificity of the test is >95% and >85%, respectively. A comparison with the Danish mix ELISA will be conducted.
2. The survival and transmission of *Salmonella choleraesuis* was investigated. Feces was collected from swine infected with *Salmonella choleraesuis* and was stored in either a dried or wet form. Bacterial survival was significantly longer in feces that had been dried. Swine also became infected following challenge by intranasal inoculation with dried feces. These data indicate that appropriate sanitation measures are critical when designing control plans in swine units and that any dried organic matter may serve as a reservoir for *Salmonella*.
3. We conducted a survey of *Salmonella* in feed transport trucks. Twenty-five culture swabs were taken from 22 trucks from 3 states; feed samples were also cultured from 17/22 trucks. Results indicate that 5/22 (22.7%) of the trucks were positive for *Salmonella* spp. *Salmonella* was isolated from approximately 4/540 swabs (0.7% sample prevalence). Three trucks had positive swabs (13.6%) and 4/22 feed samples (18.1%) were positive. Feed and swabs were positive for 2 trucks. More samples were positive from trucks containing meat, bone or fishmeal than those containing vegetable based feed which correlates with an FDA report. Four serotypes were identified and 3/4 are listed in the top 20 serotypes isolated from humans. These data indicate that while the sample prevalence of *Salmonella* in feed trucks is low (0.7%) the overall contamination rate for feed trucks is much higher (22.7%). Additionally, it may be important to monitor levels of *Salmonella* in feed transport trucks and devise methods for sanitizing between loads.
4. The carrier state of *Salmonella choleraesuis* in swine has been characterized. We have determined that route of inoculation impacts duration and magnitude of shedding, dose impacts on development of the carrier state, and shedding of *Salmonella* from naive animals exposed to a challenge group occurs within 24 h post-exposure.
5. Segregated early weaning was investigated as a means to reduce/eliminate vertical transmission of *Salmonella*. *Salmonella* isolations decreased under high health conditions.
Future studies include:

1. Participation with FSIS, APHIS, and FDA to generate baseline antimicrobial susceptibility data from *Salmonella* isolates which have been recovered from several NAHMS and FSIS surveys. These efforts are being coordinated for use in a national monitoring surveillance program with the FDA, CVM, APHIS, and CDC.
2. Monitoring swine on several farms from farrow to finish to determine when pigs become infected with *Salmonella* and if/when they clear *Salmonella*.
3. The interaction between PRRS/ *Salmonella*.
4. Role of alveolar macrophages in infection.
Salmonella Immunity: Development of a Neutrophil Phagocytosis Assay and Stress Model in Swine

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Our laboratory is interested in the immunomodulation of porcine defense mechanisms against bacterial intracellular pathogens such as Salmonella typhimurium and S. choleraesuis. Past studies indicate that levels of serum tumor necrosis factor-α (TNF-α) increase after intranasal challenge with S. typhimurium but not after oral inoculation. Challenge with S. choleraesuis has no effect on serum TNF-α concentration in the blood, regardless of route. Route of inoculation with S. choleraesuis has been shown to affect levels of lymphocyte proliferation. Both oral and intranasal routes of inoculation stimulate peripheral blood B-cells while the intranasal route is more effective at stimulating peripheral blood T-cells. The inoculum dose of S. typhimurium or S. choleraesuis can also play an important role in the host immune response. TNF-α concentrations in the blood are much greater after a $10^5$ S. typhimurium challenge than after a $10^4$ S. typhimurium challenge.

At high doses ($\geq 10^9$ CFU) S. choleraesuis causes signs of lymphocyte suppression, which may affect the ability of the immune system to eliminate the bacteria. Pigs administered an intranasal dose of $10^8$ CFU S. choleraesuis have similar immune responses as naturally infected animals.

As a means to identify immune dysfunctions associated with salmonellosis in swine, flow cytometry was used to measure the rate of ingestion of S. choleraesuis by neutrophils from swine inoculated with homologous organism. Theoretically, infection could cause a change or defect in the rate of uptake by neutrophils without necessarily changing their capacity for ingestion. The rate of ingestion by neutrophils does not increase until 2 days post inoculation (PI) and remains elevated at least 4 days PI. The decreased rate of uptake, or early lag period, after S. choleraesuis exposure may provide an opportunity for the pathogen to colonize and/or replicate to levels that facilitate establishment of a carrier-state or clinical infection.

The production and marketing of pigs presents a number of stressful situations which can lead to production losses and disease. We are developing a porcine stress model to study the effect of marketing stress on porcine immunity and bacterial shedding at time of slaughter. This model utilizes 2-deoxy-D-glucose (2DG), a sugar analog, to induce many of the hallmark responses associated with physiological stress. 2DG remains in the blood stream for $\geq 2$ hours which should allow for proper induction of a stress response. Intravenous and subcutaneous routes of injection look most promising based on release of endogenous blood glucose and cortisol, respectively. An understanding of the interaction between stress and immunity will provide important direction for future research into ways to reduce or eliminate Salmonella spp. and other diseases from pigs.
The Economic Impact of Reducing *Salmonella* Cross-Contamination During Transportation of Live Hogs
A Proposed Research Project

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New public and private initiatives to improve food safety are emerging in the pork industry. One potential control point for food safety hazards is during transportation of live animals. Research underway in the College of Veterinary Medicine at the University of Illinois is examining the effects of transportation stress in spreading *Salmonella* among hogs. The research proposed below will utilize the results of this ongoing work to investigate the impacts of alternative transportation methods on costs, food safety, and transportation flows in the pork industry.

The research has three specific objectives: 1) to develop a map of the existing transportation flows, transport methods, and associated marketing procedures in the hog industry, with a focus on the Midwest; 2) to construct a linear programming model that determines optimum transportation flows in the Midwest, and compare the model results to current transportation flows for hogs, to understand the existing economic and operational determinants; 3) to determine the changes in transportation flows that would be associated with reducing the *Salmonella* contamination risk prior to processing.

To accomplish objective 3, the model will incorporate the effects of changes in transportation costs associated with adoption of stress reduction measures. Such measures will include altering transportation methods, medication of animals before shipment, or limiting distance traveled. These changes might be adopted differentially by producers, depending upon their distance from processors and the constraints on contamination imposed by different kinds of processors. The model will determine the impact of such changes on market flows and the competitive position of producers in different locations or with different initial levels of contamination.

This research will utilize data from the Census of Agriculture and of Manufactures to create a map of transportation flows. If possible, the base model will draw on work already carried out by USDA/ERS in cooperation with USDA/GIPSA to model hog procurement in the eastern corn belt. Data from USDAAPHIS NAHMS will be used to understand the relationships among on-farm contamination, distance transported, method of transportation, marketing channel, and farm characteristics.

The results will be of use to public policy makers in identifying cost-effective contamination control policies and feasibility of policy choices, and to private firms and industry organizations in understanding the implications of new food safety initiatives for production and marketing. For example, the results might reveal whether there would be incentives for locating new production or processing facilities more closely together or whether there would be greater incentives for farm level control of contamination when hog production is more distant from processing.
A Discussion of an Epidemiologic and Economic Consideration in HACCP Evaluation: An Application of Salmonella

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This research focuses on conceptualizing the potential impact of good management practices and the associated economic costs and benefits of those practices aimed at reducing Salmonella. The aim is for feedback on ideas about systems identification, possible critical control points (CCPs), potential pathogen tests (serological vs. culture) and levels, identified test points, and potential benefits, etc. Analysis will focus on two levels, animal production (pre-harvest) and slaughter/processing (post-harvest). A primary objective will be identification of effective intervention strategies for reducing Salmonella.

Flowcharts have been developed for both animal production systems on farm and at the slaughter/processing plant. Test points and CCPs to detect presence of Salmonella have been tentatively identified for both levels. A Delphi technique was used to determine the initial critical control points and test points.

The systems flowcharts at the pre-harvest level depict pig movement within different farm management systems (one-site, two-site, and three-site swine production management systems with an all-in all-out practice) and include transportation of animals. Systems flowcharts at the post-harvest level show that the majority of CCPs and test points are at the front end of the slaughter/processing cycle, and include test points for Salmonella at the final stage of fabrication. The slaughter facility will include pigs from multiple sources which will be commingled at slaughter. This systems approach allows for evaluation of good management practices based on the prevalence levels of Salmonella at the beginning of the slaughter cycle as compared to the end of fabrication.

Culturing vs. rapid test (MIX ELISA) will be evaluated accordingly to determine: costs differences, labor requirements, turnaround time, ease of collection, impact on line speed, capital requirements, etc. Advantage and disadvantages of each method and, perhaps, identification of a balanced approach based on information and knowledge gained will be a focus.

Economic implications or costs of management interventions at identified CCPs for the animal production level and the slaughter plant level will be determined. These costs will be compared to the potential benefits for Salmonella reduction. For producers, potential comparisons will be intervention costs vs. performance changes such as: average daily gain or time to market, pounds produced per square foot, or potential changes in animal quality and value.

For producers/processors intervention costs at identified CCPs will be compared to benefits for products with reduced or low levels of Salmonella. Benefits may be a premium price for products with reduced Salmonella, reduced product losses due to extending the product shelf life, product appearance, etc. Packers/processors will be surveyed to obtain feedback on those potential benefits. Knowledge gained will be incorporated into good management practice recommendations. Information on costs and benefits can provide insight into potential premiums or discounts based on Salmonella levels in swine and pork products. It will allow determination of added value for reduced Salmonella levels.
Control of \textit{Salmonella} Virulence in the Natural Host

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The long term goal of this research is to understand the mechanisms that govern \textit{Salmonella} virulence in the natural host. Much of the genetic and pathogenesis studies have focused on using the murine model of salmonellosis. Although the results from using the model have been useful, there have been several cases where mutants of \textit{Salmonella} not showing virulence in mice have been virulent in the natural host and visa versa. Therefore, virulence may be mediated by the host to some degree. We have chosen \textit{Salmonella choleraesuis} which is host-adapted to swine, to study particular genes that may be crucial in causing disease. Specifically, we have begun a project to evaluate several mutants of \textit{Salmonella choleraesuis} and test them for virulence. We will use strains containing separately or in pairs the following mutations: \textit{sly}, \textit{rpoS}, and \textit{spvR}. The \textit{sly} and \textit{rpoS} are chromosomally encoded genes that are involved in the pathogenesis of \textit{Salmonella}. The \textit{sly} gene is a cytolsin that has been shown to be required for virulence in mice. Preliminary studies indicate that it may also be required for virulence in swine. Swine that have been infected with a \textit{sly} mutant of \textit{Salmonella typhimurium} do not develop disease. The hypothesis for the function of this gene is that \textit{sly} is one of the genes that is key for the survival of \textit{Salmonella} in macrophages. We also propose that mutants of \textit{Salmonella} that cannot survive in professional phagocytic cells should not persist in the g.i. tract and contribute to stress induced reactivation. Mutants that are unable to cause disease may be good live vaccine candidates. The \textit{rpoS} gene is the alternative, starvation-stationary sigma factor that is responsible for the global switch of gene expression as cells enter stationary phase growth or are starved for nutrients. We feel that both of these conditions exist in the host as \textit{rpoS} mutants of \textit{Salmonella typhimurium} are avirulent in mice. Evaluating the virulence of this mutant in \textit{Salmonella choleraesuis} infected swine will aid in the understanding of the conditions \textit{Salmonella} must endure in the host. Non-typhoidal \textit{Salmonella} serovars that are host adapted may contain a large virulence plasmid. Of the strains that contain this plasmid, all contain the \textit{spv} operon (\textit{Salmonella} plasmid virulence). These genes are controlled by \textit{spvR}, which has been shown to be a transcriptional regulator and a DNA binding protein. \textit{SpvR} only has a regulatory effect on the \textit{spv} genes and no chromosomal genes that have been detected. In \textit{Salmonella dublin}, \textit{spvR} mutants are avirulent in calves as they are in mice. An \textit{spvR} mutation will be introduced into \textit{Salmonella choleraesuis} and the effect on virulence determined in swine.

These studies are all designed to determine the genetic basis of host adaptation of \textit{Salmonella}. Only by using specific mutations in isogenic pairs can a role be assigned to the function of the gene products. Vaccine development is the ultimate goal of the research, using defined mutations to create strains that are avirulent, highly immunogenic, protective, and do not linger or persist in the environment.
Use of Defined Competitive Exclusion Cultures to Enhance Colonization Resistance to Enteric Pathogens

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¹USDA/ARS, Food Animal Protection Research Laboratory
College Station, Texas 77845 and ²USDA/ARS, National Animal Disease Center, Ames, IA 50010

During the past several years our laboratory has conducted research towards developing defined competitive exclusion cultures that enhance colonization resistance against salmonellae in baby chicks. Previously in our laboratory it was shown that 10-day-old broiler and layer chicks that were fed diets containing 5-10% lactose provided either in the feed or in water from day-of-hatch were significantly more resistant to Salmonella typhimurium, and S. enteritidis cecal colonization than control chicks not provided a diet supplemented with lactose. Additionally, resistance against salmonellae cecal colonization was further enhanced in treatment groups provided dietary lactose in combination with an undefined mixture of anaerobic bacteria (i.e. undefined competitive exclusion culture or Nurmi culture) originally obtained from the ceca of adult broiler chickens maintained on a diet containing lactose. In order to make a defined competitive exclusion culture that was efficacious in enhancing colonization resistance against salmonellae, we cultured cecal contents obtained from adult broilers maintained on a unmedicated diet containing 5% lactose in a continuous-flow (CF) culture apparatus (i.e. chemostat), that was maintained at parameters that would best represent the cecal environment. Two different CF-cultures were developed from the same initial inoculum with the difference being that one CF-culture medium contained lactose and the other did not. From these CF cultures two different defined cultures were developed (CF1 and CF2) that were efficacious in enhancing colonization resistance against S. typhimurium in broilers and S. enteritidis in layers, when used in combination with diets containing lactose. Although both of these CF cultures significantly protected day-old chicks against salmonellae colonization production economics dictated that a defined culture be developed that was efficacious in controlling salmonellae cecal colonization in the absence of dietary lactose. A third CF-culture was developed employing the same techniques used for the previous CF-cultures with notable exceptions. The original inoculum came from adult broilers fed an unmedicated diet that did not have lactose included in the formulation, and the culture medium also had no lactose. From this inoculum a CF-culture containing 29 different bacterial isolates was developed (CF3). This CF-culture has been shown under experimental conditions to significantly protect against S. typhimurium and S. enteritidis colonization and in a large commercial field trial to significantly reduce salmonellae colonization in adult market-age broilers. Other research with CF3 has shown that chicks provided the culture at day-of-hatch have greater than a 100-fold increase in cecal anaerobic CFU at three-days-of-age compared to untreated controls. Additionally it has been shown by electron microscopy that a large proportion of this increase occurs on the cecal mucosal epithelium. The results of this rapid establishment of a cecal bacterial ecosystem is a significant increase in cecal total volatile fatty acids, with the most notable increase in propionic acid. Over several studies the correlation between the level of cecal propionic acid in three-day-old chicks and salmonellae colonization has been extremely high and we now use this physiological response as a indicator of CF3 culture establishment and a predictor of efficacy in laboratory experiments. This increase in propionic acid was also observed in the commercial field trial mentioned previously. This technology has been licensed to an industry partner whom we have a cooperative research and development agreement (CRADA) with and is currently in the FDA process and a large scale commercial fermentation facility is under construction. Currently we are employing the same techniques used to develop CF1, CF2, and CF3 to develop a defined CF-culture that will be
efficacious in reducing salmonellae colonization in swine. This research is being conducted under a CRADA with our industry partners and in cooperation with the USDA/ARS Salmonellosis in Swine research unit at Ames Iowa. Presently a CF- culture has been developed and is being used in laboratory experiments to determine if this type of pathogen intervention strategy can also be used in the swine industry to aid in the reduction of enteric pathogen colonization in the swine gut.
Perinatal Vaccination Against Salmonellosis

Ted T. Kramer, DVM, PhD
Iowa State University, Ames, IA

Dr. Ted Kramer has investigated various aspects of swine salmonellosis since 1980. These investigations consisted of a search for virulence criteria, affecting the humoral and CMI immune systems (1-4). An immunohistochemical assay was developed for detecting Salmonella in swine tissues (5). The role of iron and iron-binding proteins was investigated in salmonellosis of pigs (6,7). A search was made for spontaneous mutants and Tn10 induced mutants as potential vaccine strains (8). A porcine neutrophil survivor clone was recognized as a good vaccine candidate against swine paratyphoid (9,10). The effect of S. choleraesuis and of LPS on physiologic and immunologic liver functions was investigated (11). A search was made for a suitable indirect ELISA assay to detect carrier pigs (12).

Recently, the factors affecting vaccination of perinatal pigs with an experimental Aro A vaccine was investigated (Kramer and Stocker, manuscript in preparation). It was found that the presence or absence of maternal (colostral and milk) antibodies was the most important factor affecting perinatal immunity and the success of vaccination. The importance of this observation is twofold: (1) it points to the importance of preexisting maternal antibody in active immunization against salmonellosis of newborn pigs; and (2) it suggests that humoral immunity may be important in swine salmonellosis, contrary to the generally held view that immunity to salmonellosis is primarily cell-mediated. These points will be illustrated in the presentation.

Salmonella Surveillance in U.S. Swine Herds

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Previous Research Efforts

Past research has focused on *Salmonella choleraesuis* virulence determinants and the interaction of this organism with the porcine immune system. Emphasis was placed on interaction of virulent *S. choleraesuis* field isolates with neutrophils and macrophages. Results suggested a correlation between relative bacterial virulence and the organisms ability to interfere with phagocyte ingestion, inhibit superoxide anion production, and overall intracellular survivability. A 40 kDa outer membrane protein was identified and determined to be involved with bacterial translocation and eukaryotic cell invasion.

Subsequent work with Dr. T. Kramer evaluating *S. choleraesuis* repeatedly passed through porcine neutrophils led to the discovery of an avirulent live *S. choleraesuis* isolate. I was involved in the characterization of this isolate and subsequently the commercialization of an avirulent live vaccine (NOBL Laboratories; SC-54). Research by myself as well as others has demonstrated the vaccine is an effective aid in the prevention of porcine salmonellosis caused by *S. choleraesuis*. Research has demonstrated that pigs vaccinated intranasally at 3 weeks of age are protected following virulent challenge from 2 to 16 weeks post-vaccination. Research by NOBL and a collaborative scientist has consistently demonstrated that vaccinated animals have reduced numbers of infected organs following virulent challenge exposure. In some studies the relative level of *Salmonella* per/gm of tissue has been reduced and there are some indications that fecal shedding of *S. choleraesuis* is reduced in vaccines compared to non-vaccinates.

A collaborative effort has also been completed evaluating PCR detection methods and a unique primer set for *Salmonella* detection in both field samples and pure culture.

Current Research Efforts

Current efforts are focused on *Salmonella* reduction and monitoring systems to be used by practitioners and possible role in a HACCP/Salmonella Reduction Program. Efforts include:

1. Vaccination of pigs at day 1 of age to reduce pre-weaning infection.
2. Vaccination of pigs and potential cross protection/reduction of *Salmonella* serotypes other than *S. choleraesuis*. Initial work will focus on *S. typhimurium*.
3. Implementation, validation, and evaluation of the Danish *Salmonella* ELISA in U.S. pigs.
4. Correlation of Danish ELISA results with Salmonella isolation at slaughter. We will also evaluate vaccinated and non-vaccinated pigs to determine potential effects of vaccine use under field conditions.

Farm-level Risk Factor Assessment for Infection with *Salmonella* spp.

Peter B. Bahnsen¹, DVM, PhD, and Paula J. Fedorka-Cray², PhD

¹University of Minnesota, St Paul, MN 55108 and ²National Animal Disease Center, Ames, IA 50010

**Current Research**

We are focusing on the factors that predispose to *Salmonella* spp. infection in commercial pig production systems. Our current project aims to accomplish two primary goals. First, we will define the herd-level and pig-level prevalence for *Salmonella* infection. Second, we will quantify the farm-level risk factors for *Salmonella* herd- and pig-level prevalence. The determination of *Salmonella* infection is species and serotype specific. Twenty-five pigs will be sampled on the farm and 15 at slaughter from each of 70 herds that participate in an ongoing slaughter monitoring project (PigMON). Risks will be assessed by a survey of farms at the time of the slaughter inspection.

Information is being collected regarding farm management, facilities, equipment, and feed, among others. Multivariate categorical analysis will be used to quantify the relative odds of infection with the presence of potential risk factors. We expect to accurately define herd- and pig-level prevalence of *Salmonella* spp. among commercial farms that use the PigMON system, and to provide a comprehensive assessment of farm-level risk factors. This project is expected to provide the important information on hazards to allow for the development of farm-level HACCP protocols.

**Preliminary Results**

As of March 1, 1996, we have collected samples from 32 farms; serotype data is available from 21 farms. A total of 14 serotypes have been isolated. The five most common isolates overall have been *S. derby*, *S. agona*, *S. typhimurium (copenhagen)*, *S. anatum*, and *S. schwarzenegger*. Both the farm- and pig-level prevalence of *Salmonella* spp. has been higher in cecal contents collected at the slaughter plant than in feces collected at the farm. Among all 1591 samples for which culture results are known, 127 (8%) are positive. The farm level survey shows diversity in production systems, facilities, and management characteristics. Sample size is as yet inadequate to test for farm-level risk factors.

**Future Priorities**

Development of systems to reduce or eliminate human health hazards will require sound scientific research. We plan to focus efforts on the development of commercially applicable, science based strategies to reduce food safety hazards in production agriculture.
Epidemiology of *Salmonella* in Swine Production Systems in North Carolina

Peter Davies, Morgan Morrow, Frank Jones, John Deen, Maria Correa, Julia O'Carroll, Julie Funk

*College of Veterinary Science, College of Agriculture and Life Sciences North Carolina State University*

**SYNOPSIS OF WORK**

Background: Program initiated in 1994 when Dr. Davies joined the College of Veterinary Medicine, NCSU.
Long term goal: to make a major contribution to knowledge of the epidemiology, in swine production systems, of foodborne pathogens of humans.

**Projects and Sources of Funding (Title, Source, Time-frame, status):**

1. **Effects of emerging swine production systems on the prevalence of *Salmonella* and *Toxoplasma gondii* in pigs. NPPC. July 1994-June 1995. (completed 9/95).**

   Cross-sectional study of the prevalence of *Salmonella* spp. (and *Toxoplasma/Trichinenella*) in market-age swine in different production systems in North Carolina. Twenty nine herds were included in the study. Herds purposely selected by overall production system. Group A: 14 finisher barns using all-in/all-out management by building. Seven farms with fully slotted floors (SF), 7 with solid floors and open flush gutters (OFG). Group B: 14 farrow-to-finish herds with continuous flow finishing barns. Seven (+1 university herd) herds-total confinement, 7-finishing pigs some access to outside accommodation (3 on dirt, 3 on concrete, 1 on pasture).

   Twenty-four of 29 farms were positive (at least one *Salmonella* isolated). Salmonellae were isolated from 594 (26%) of 2326 individual fecal samples. Among positive herds, prevalence ranged from 2% to 88% of fecal samples. The 6 serotypes found on the most number of farms were *S. derby*, *S. worthington*, *S. typhimurium*, *S. typhimurium (copenhagen)*, *S. heidelberg*, and *S. mbandaka*. Several of these serotypes are among the most common isolates from cases of clinical disease in humans in the U.S. *Salmonella choleraesuis*, the most common isolate from clinical cases in swine in the U.S. was isolated from only 6 samples on 2 farms.

2. **Longitudinal studies of *Salmonella* infection of swine in multiple-site production systems. North Carolina Pork Producers Association/NPPC. 1995-98.**

   Objectives: 1) identify points in the pork production cycle when infection with *Salmonella* occurs 2) identify likely key sources of infection 3) evaluate the stability of these observations over time within herds. Sampling of pigs will focus on repeated sampling from individual animals over time. In breeding herds, one group of newly arrived gilts will be repeatedly sampled over two years. At nursery and finisher sites, observations will be replicated in 3 groups raised in the same facilities. Intensive sampling of all animals in randomly selected pens combined with random sampling from other pens within the same buildings and in other buildings on the same sites. Also feed, water and environmental samples (including trapped rodents and liquid from flushing tanks) at each visit.

Field work initiated - no results available
3. Other initiatives (unfunded):
   a) Evaluation of delayed secondary enrichment and delay in processing on the recovery of Salmonella from fecal samples (being conducted)

4. Future Objectives
   1) Evaluation of epidemiological markers for studying Salmonella in swine populations
The Presence and Prevalence of *Salmonella*, *Campylobacter* spp. and O-serotypes of *E. coli* in Swine Raised Under Differing Management Schemes

J.N. Nielsen and J.A. Patterson*
Department of Animal Sciences
Purdue University,
West Lafayette, IN 47907-1151

There is increasing demand by the public for assurance of an economical, high quality and safe food supply. Microbial contamination of foods at slaughter is of increased interest, because one of the primary sources of carcass contamination is from feces. Thus, from a food safety perspective, a HACCP critical control point is the late finishing stage and how pigs are handled from farm to slaughter. Segregated early weaning systems produce pigs in fewer days to market than continuous flow systems, with higher health status and fewer respiratory pathogens. Feed is withheld from pigs during shipping, however, fasting reduces the inhibitory effect of the normal intestinal microflora and could potentially result in increased fecal pathogen levels. Thus, the objectives of this research project were: 1) compare multi-site SEW versus continuous flow rearing of swine on incidence of fecal pathogens of human health significance, 2) compare the effect of 24 hour fasting versus full feeding on the intestinal pathogen concentrations at slaughter with pathogen positive pigs, 3) assess the incidence of carcass contamination in pathogen positive and representative control swine.

One hundred twenty pigs were placed in segregated early weaning nursery facilities followed by placement into a cleaned, disinfected curtain-sided grower finisher unit, where they were reared until market weight of 240-260 lb. was reached. Littermate pigs (120) were kept with their dams until 28-30 days of age, at which time they were moved to an all-in, all-out nursery, followed by introduction into a continuous flow finisher building at 8 weeks of age. This group of pigs remained in the continuous flow finisher unit, interspersed with pens of pigs of varying ages, until they reached market weight. All pigs received Tylan® during the finishing phase. Rectal fecal specimens were collected from 1 week to 1 month prior to slaughter. The fecal specimens were cultured within 4 hours of collection for the presence of *Salmonella*, *Campylobacter* and O-serotype *E. coli*. Only four pigs tested positive for *Salmonella* during the later part of the finishing stage. However, these four pigs were from the high health status group. All pigs were negative for *Campylobacter* and O-serotype *E. coli*.

The *Salmonella* positive pigs, as well as an additional 18 *Salmonella* negative pigs were divided into two groups. One group was fasted for 24-30 hours prior to slaughter, while the second group was full-fed up to 4-6 hours prior to slaughter. Pigs were rectally sampled prior to and at the end of the fast. These pigs were followed to the slaughter plant and the chilled carcasses were swabbed at the shoulder, loin and ham. The samples were plated for the presence of pathogenic bacteria. All swabs collected from the carcasses were culturally negative for *Salmonella* and *Campylobacter* spp. and O-serotype *E. coli*.

In summary, 4 of 226 pigs sampled in this study were positive for *Salmonella* and all of the positive pigs were found in the high health status group of pigs. Only 1 of the 4 pigs initially testing positive for *Salmonella* remained positive at more than 1 sampling period, suggesting that shedding is periodic. With the small number of positive pigs, it was impossible to determine if fasting increased pathogen numbers.
Studies on *Salmonella* Infections in Pigs with Emphasis in Food Safety Applications

David H. Baum¹, D. L. Hank Harris¹, Bent Nielsen², Paula J. Fedorka-Cray³, M. B. Roof⁴, J. Torrison⁵, and G. BeVier⁵

¹Iowa State University, Ames, IA, ²Danish Veterinary Laboratory, Copenhagen, Denmark, ³NADC, Ames, IA, ⁴NOBL Laboratories, Ames, IA, and ⁵PIC, Inc, Franklin, KY

During the past year, studies have been conducted in the following areas:

1. the seasonal variations in environmental fecal *Salmonella* in pigs prior to shipment for slaughter
2. identification of groups of pigs that might be at risk for becoming infected with *Salmonella*
3. comparison of culture and the Danish MIX-ELISA for detection of *Salmonella* on pig farms
4. efficacy of SC54 vaccination of pigs at one day of age
5. the comparison of culture and the Danish MIX-ELISA for the detection of *Salmonella* in carcasses at slaughter
6. the impact of *Salmonella* infections on performance

Preliminary data indicate the following for each study:

1. There appears to be a seasonal pattern of shedding of *Salmonella* from pigs. There also appears to be different serotypes of *Salmonella* shed throughout the year.
2. The ELISA is faster, as effective, and less expensive for detecting groups of pigs that are *Salmonella*-positive.
3. Pigs can be vaccinated at one day of age and be protected against challenge to *S. choleraeuis* at 5 weeks of age. Pigs vaccinated at 21 days of age do not have significantly higher ELISA titers to *Salmonella* than those pigs that are not vaccinated.
4. The ELISA also detects those groups of pigs that are positive by culture at slaughter.
5. There appears to be a reduction in growth performance of pigs if the pigs are infected with *Salmonella*.
6. Risk factors have been identified and associated with the development of infections by *Salmonella* in pigs. These risk factors can be used to predict whether *Salmonella* infections will develop.

Current projects:

1. cross protection of SC54: whether pigs vaccinated with SC-54 are protected against infection by *S. typhimurium*
2. whether the ELISA can be used as a diagnostic tool for *S. choleraeuis* infections
3. getting more data to substantiate risk factors for groups of pigs to develop *Salmonella* infections
Report on *Salmonella* Studies of the Food Safety Consortium

Dr. George W. Beran  
*Iowa State University, Ames, IA 50011*

The meat and meat products research program integrated in the consortium of the University of Arkansas (UA), Iowa State University (ISU), and Kansas State University (KSU) is functioning in safety research predominantly on poultry (UA), pork (ISU), and beef (KSU). Studies on prevention and control of *Salmonella* have been a special emphasis of the Food Safety Consortium.

The goals of the Consortium are to conduct research for:

- a. Development of technology for rapid identification of infectious agents and toxins within the processing and distribution chains.
- b. Development of a data base and statistical framework to evaluate the extent of potential health risks posed by the contamination of the animal product food chain by infectious agents, chemical/drug residues and toxins.
- c. Analysis of the animal product food chain to determine the most effective points at which intervention could occur to control or prevent a microbiological or chemical hazard cycle.
- d. Development of techniques to monitor processing and distribution of food products of animal origin to detect potential microbiological or chemical hazards.
- e. Development of costs and benefits of risk assessment and interdiction actions in hazard reduction/control.

Summaries of major studies entirely or partly focused on *Salmonella* in the three Consortium Universities are presented here, identified by the principal sites of the research. Reports of all of these studies, including all of the investigators who performed them are available in the annual Food Safety Consortium Reports which are gladly supplied upon request.

*Salmonella* studies will be an increasing priority during 1996-1998. A large study on the epidemiology of *Salmonella* infections in swine at farm level followed through the pork chain to *Salmonella* contamination of pork and pork products at consumer level is in pilot phase at Iowa State University and National Animal Disease Center and will move to full scale in 1996.

A study on hot water rinses on *Salmonella* contaminated swine carcasses will be continued in 1996-1997. Prevalence studies on *Salmonella* as well as other pathogenic bacteria in ground pork from suppliers varying from unfrozen preground product to fresh ground on site product will be continued at Iowa State University.

Beef carcass treatment by steam/vacuum and other methods to reduce *Salmonella* and other pathogens are a major research focus at Kansas State University. Extensive studies on *Salmonella* and other pathogen control both in industry and the laboratory will be focused at the University of Arkansas.

*Salmonella* is the meat borne pathogen of major priority for control at this time. With the Centers for Disease Control and Prevention identifying human salmonellosis in the United States as causing nearly 2 million human illnesses, between 1 and 2 thousand deaths and economic costs between 1 and 2 billion dollars, *Salmonella* control has our attention.
Prevalence of *Salmonella* spp. in Survey Herds

National Animal Disease Center

715 fecal samples collected from 15 swine herds in 15 states (pre-survey study) and 4,977 samples from 100 feedlot cattle herds in 13 states (full survey) were cultured for *Salmonella* spp. in Tetrathionate broth 48 hours, then in Rappaport medium (T48-R); in tetrathionate broth 48 hours (T-48); and in GN Hajna broth 24 hours; then in Rappaport medium (GN-R).

<table>
<thead>
<tr>
<th>Species</th>
<th>Tests</th>
<th>No.</th>
<th>Tested</th>
<th>Percent Positive for <em>Salmonella</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Swine</td>
<td>Herds</td>
<td>15</td>
<td>40%</td>
<td>Total 83% T48-R 50% GN-R 33%</td>
</tr>
<tr>
<td></td>
<td>Animals</td>
<td>715</td>
<td>3.8%</td>
<td>93% 78% 30%</td>
</tr>
<tr>
<td>Cattle</td>
<td>Herds</td>
<td>100</td>
<td>38%</td>
<td>97% 71% 55%</td>
</tr>
<tr>
<td></td>
<td>Animals</td>
<td>4,977</td>
<td>5.5%</td>
<td>77% 50% 37%</td>
</tr>
</tbody>
</table>

ELISA Tests for *Salmonella* Infection in Swine

Iowa State University

An indirect (antiglobulin) ELISA test developed at Iowa State University, serum ELISA and tissue extract ELISA tests developed in Denmark, and fecal culture have been studied for identification of *Salmonella* infections in experimental and field swine herds.

Experimental *S. choleraesuis* Infections

<table>
<thead>
<tr>
<th>Inoculum</th>
<th>Uninoculated</th>
<th>$10^3$</th>
<th>$10^6$</th>
<th>$10^8$</th>
<th>$10^9$</th>
<th>$10^{10}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of Pigs</td>
<td>3</td>
<td>5</td>
<td>5</td>
<td>4</td>
<td>7</td>
<td>4</td>
</tr>
<tr>
<td>ELISA assays</td>
<td>All Neg</td>
<td>1 pos</td>
<td>4 pos</td>
<td>All pos</td>
<td>All pos</td>
<td>All pos</td>
</tr>
<tr>
<td>Culture assays</td>
<td>All Neg</td>
<td>All Neg</td>
<td>All pos</td>
<td>All pos</td>
<td>-</td>
<td>All pos</td>
</tr>
</tbody>
</table>

Field Infection Studies

Danish MIX-ELISA was as effective as culture for identifying finishing units with infected swine.

In a swine herd with history of clinical *Salmonella choleraesuis* infection, the indirect serum ELISA test demonstrated that segregated early weaning reduced the prevalence but did not ensure elimination of infections.

---------------
Growth of *Salmonella typhimurium* in Universal Pre-enrichment Media with Supplements

**Kansas State University**

*Salmonella* Generation Time

<table>
<thead>
<tr>
<th>Condition</th>
<th>Time (minutes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Universal pre-enrichment media (UP)</td>
<td>22.9</td>
</tr>
<tr>
<td>UP plus 0.4 units/ml oxyrase</td>
<td>16.5</td>
</tr>
<tr>
<td>UP plus 1.0 μg/ml ferrioxamine E</td>
<td>10.5</td>
</tr>
<tr>
<td>UP plus oxyrases and ferrioxamine E</td>
<td>16.0</td>
</tr>
</tbody>
</table>

------------

**Reported Surveys for *Salmonella* spp. Prevalence on Meat**

**Iowa State University**

<table>
<thead>
<tr>
<th>Source</th>
<th>Carcass</th>
<th>Fresh Meat</th>
<th>Organ Meat</th>
<th>Ground Meat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Summary of Reports on Pork</td>
<td>16.2%</td>
<td>14.7%</td>
<td>30.0%</td>
<td>40.3%</td>
</tr>
<tr>
<td>Summary of Reports on Beef</td>
<td>1.0%</td>
<td>7.8%</td>
<td>-</td>
<td>46.0%</td>
</tr>
<tr>
<td>Summary of Reports on Poultry</td>
<td>47.4%</td>
<td>41.9%</td>
<td>52.7%</td>
<td>-</td>
</tr>
<tr>
<td>Three Iowa Plant Study on Pork</td>
<td>0.4-4.4%</td>
<td>0-0.7%</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Fourteen Ground Pork Companies</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>15.6%</td>
</tr>
<tr>
<td>Food Services of 3 Care Facilities:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pork products post-thawing</td>
<td>-</td>
<td>0% of 12</td>
<td>-</td>
<td>0% of 6</td>
</tr>
<tr>
<td>Pork products at end of service</td>
<td>-</td>
<td>0% of 12</td>
<td>-</td>
<td>0% of 6</td>
</tr>
</tbody>
</table>

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**Effectiveness of Isolated Weaning and Rearing of Pigs from Three Field Herds**

**National Animal Disease Center**

Swine herds on three farms were sources of 10-21 day old weaning pigs which were then raised in isolation and monitored by culture for *Salmonella* infections to 6 weeks of age.

<table>
<thead>
<tr>
<th>Farm</th>
<th>Trials</th>
<th>Culture Positive Pigs by Trial Number</th>
<th><em>Salmonella</em> spp.</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1-4</td>
<td>1:0/59 2:19/78 3:15/77 4:0/71</td>
<td><em>S. derby</em></td>
</tr>
<tr>
<td>B</td>
<td>5-9</td>
<td>5:0/7 6:7/74 7:0/20 8:0/21</td>
<td><em>S. mbandaka</em></td>
</tr>
<tr>
<td>C</td>
<td>10-16</td>
<td>10:0/6 11:0/6 12:0/25 13:0/35</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>14:0/40 15:0/20 16:0/40</td>
<td>-</td>
</tr>
</tbody>
</table>

52
Effectiveness of Adding Aluminum Sulfate to Broiler Litter to Reduce Pathogens

University of Arkansas

Aluminum sulfate was added to broiler litter in ten farm production units and the broiler chickens were monitored at the farms and slaughter plants for pathogenic bacteria.

Prevalence of *Salmonella* was significantly reduced in treated litter, but at the end of commercial slaughter, no differences were detected on carcasses of broilers raised on untreated litter.

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*Salmonella* Reduction on Poultry Carcasses and Chill Water

University of Arkansas

Freshly butchered chicken carcasses were inoculated with 10<sup>6</sup> *Salmonella typhimurium*, experimentally treated in chill water and cultured.

Addition of 1% trisodium phosphate, sodium carbonate or sodium bisulfate to chill water reduced *Salmonella* titers by 0.26-0.42 logs on carcasses and by 90-95% in chill water.

Treatment of the carcasses in chill water with these chemicals plus low voltage pulsed electric current reduced *Salmonella* titers by 0.53-1.80 logs on carcasses and below detectable levels in chill water.

Electron microscopic studies showed that electrical treatment disrupted *Salmonella* cytoplasmic membranes and scattered cytoplasm into condensed particles the cells.

----------

*Salmonella* Reduction on Chicken Carcasses by Combination Treatments

University of Arkansas

In 3 trials, chicken carcasses scalded at 56 and 600°C were each defeathered, then sprayed with 10<sup>7</sup> CFU *Salmonella typhimurium* and incubated 30 minutes. They were then treated by dipping in 1% trisodium phosphate or/and application of 50 volt 100 Hz electric current and cultured.

Following 56°C scalding and defeathering, 6.6-7.5 logs *Salmonella* attached per carcass, a mean increased attachment of 0.5 logs following the higher temperature scald.

Treatment with trisodium phosphate reduced *Salmonella* numbers on carcasses scalded at 56°C by 0.4-0.6 logs and on those scalded at 60°C by 0.2-1.4 logs, a mean greater reduction of 1.0 logs on poultry treated after the higher temperature scald.

Treatment with trisodium phosphate and electric current reduced *Salmonella* numbers on carcasses scalded at 56°C by 0.4-0.7 logs and on those scalded at 60°C by 0.5-1.8 logs, a mean reduction of 0.8 logs on poultry treated after the higher temperatures scald.

----------
**Salmonella Reduction on Poultry Carcasses by Chemical Sprays**  
**University of Arkansas**

Freshly butchered chicken carcasses were inoculated with 10^6 *Salmonella typhimurium*, experimentally sprayed with food grade chemicals and cultured.

Spraying with 0.1% cetylpyridinium chloride, 1% lactic acid, 5 and 10% trisodium phosphate, and 5 and 10% sodium bisulfate at 30 psi for 30 seconds reduced *Salmonella* titers by 0.59-1.57 logs.

Increasing spraying time to 90 seconds reduced titers an additional 0.26-2.13 logs, but increasing pressure to 50-120 psi did not significantly further reduce *Salmonella* titers.

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**Salmonella Reduction on Chicken Skin by Cetylpyridinium Chloride**  
**University of Arkansas**

Chicken skin and whole carcasses inoculated with 10^5-10^7 *Salmonella typhimurium* were sprayed or dipped in 0.1% cetylpyridinium chloride and cultured.

Spraying contaminated skin for 1-3 minutes at 15-50°C reduced *Salmonella* titers by 0.9-1.7 logs. Longer chemical contact and higher temperatures were moderately but not always significantly more effective.

Immersion of contaminated skin yielded *Salmonella* titer reductions of 1.0-1.6 logs, not significantly different from spraying.

Spraying whole inoculated carcasses 30 seconds plus 0-3 minutes contact time reduced *Salmonella* titers 0.45-0.77 logs.

Immersion of whole inoculated carcasses 1-3 minutes reduced *Salmonella* titers 2.35-3.84 logs.

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**Salmonella Reduction on Commercial Turkey Carcasses by Chemical Treatments**  
**University of Arkansas**

Fresh or thawed turkey carcasses treated or not treated at the same processing plants with trisodium phosphate were cultured for *Salmonella* spp. Freshly butchered turkeys were also chilled in water with 1-3 ppm chlorine dioxide or 25-40 ppm chlorine and cultured for *Salmonella* spp.

In three experiments, *Salmonella* was cultured from 65%, 31%, and 5% of untreated turkeys and from 10%, 56%, and 30% respectively of trisodium phosphate treated carcasses.

Prevalence of *Salmonella* contamination on turkey carcasses was not significantly modified by addition of chlorine dioxide or chlorine to chill water.

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Effectiveness of Decontamination of *Salmonella* on Beef Carcasses

**Kansas State University**

Freshly butchered beef carcasses were contaminated with $10^5$ CFU/cm$^2$ of *Salmonella typhimurium* suspended in cattle feces, treated by single decontamination methods and cultured.

<table>
<thead>
<tr>
<th>Decontamination Treatment</th>
<th>Mean Titer Reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Knife trimming of visible contamination</td>
<td>2.5 logs</td>
</tr>
<tr>
<td>35°C water wash</td>
<td>1.3 logs</td>
</tr>
<tr>
<td>Vacuum followed by 54°C water spot cleaning</td>
<td>3.3 logs</td>
</tr>
<tr>
<td>15 seconds steam</td>
<td>3.4 logs</td>
</tr>
</tbody>
</table>

----------

Combination Decontamination of *Salmonella* on Beef Carcasses

**Kansas State University**

Freshly butchered beef carcasses were contaminated with $10^5$ CFU/cm$^2$ of *Salmonella typhimurium* suspended in cattle feces, treated by combination decontamination methods and cultured.

<table>
<thead>
<tr>
<th>Decontamination Treatment</th>
<th>Mean Titer Reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Knife trimmed; 35°C water wash</td>
<td>4.9 logs</td>
</tr>
<tr>
<td>Knife trimmed; 35°C water wash; 15 seconds steam</td>
<td>4.6 logs</td>
</tr>
<tr>
<td>35°C water wash; 15 seconds steam</td>
<td>4.4 logs</td>
</tr>
<tr>
<td>Vacuum with 54°C water cleaning; 35°C water wash</td>
<td>3.5 logs</td>
</tr>
<tr>
<td>Vacuum with 54°C water cleaning; 35°C water wash; 15 sec. steam</td>
<td>3.8 logs</td>
</tr>
<tr>
<td>Knife trimmed; 35°C water wash; 2% lactic acid spray; 15 sec. steam</td>
<td>5.1 logs</td>
</tr>
<tr>
<td>Vacuum with 54°C water cleaning; 35°C water wash; 5-15 sec. steam</td>
<td>4.2-5.0 logs</td>
</tr>
</tbody>
</table>

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Studies on a Newly Recognized Bacteriocin from *Bacillus subtilis*

**University of Arkansas**

A bacteriocin elaborated by a strain of *Bacillus subtilis* cultured from a Chinese fermented food was tested for bacterial inhibitory activity in vitro.

The partially purified bacteriocin was broadly inhibitory to *Salmonella* spp. and a selected group of both Gram negative and Gram positive pathogenic bacteria.

The bacteriocin was heat stable, active over a broad pH range, but was inactivated by peptidases and lipase.
Effectiveness of Irradiation on *Salmonella* Contaminated Pork

Iowa State University

Low dose (0.75-0.90 kGy) and medium dose (1.8-2.0 kGy) irradiation was applied to pork chops and sliced ham inoculated with 5-6 logs of *Salmonella typhimurium* and then held at 7°C for 7 days and at 25°C for 2 more days with cultures at 0, 7, and 9 days.

Both low dose and medium dose irradiation were effective in immediate reductions in culturable *Salmonella*, by at least 2 logs at low dose and at least 4 logs at medium dose.

Irradiation at levels tested (≤2.0 kGy) did not kill *Salmonella*, permitting resumption of growth when subjected to incubation temperature after one week storage.

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Acid and Thermal Resistance of Acid Adapted *Salmonella* spp.

Iowa State University

*Salmonella typhimurium* and *S. typhimurium* ATCC14028, *S. dublin*, and *S. heidelberg* adapted to growth at pH 5.0 were inoculated on beef rounds, which were then tested for lactic acid rinse tolerance at 1.5 and 3.0%, and for thermostolerance at 23 and 55°C.

Acid rinses were significantly more effective in inactivating acid adapted than the parent strains of *Salmonella typhimurium* ATCC14028, *S. dublin*, and *S. heidelberg* of bovine origin and equally effective against *Salmonella typhimurium* of bovine origin.

Acid adaptation significantly decreased the tolerance of *Salmonella typhimurium* and *S. dublin* of bovine origin to 55°C heat.

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Epidemiology of Salmonellosis in Children

University of Arkansas

90 children under 4 years of age with acute salmonellosis admitted to Arkansas Children’s Hospital were studied.

54% of the patients were under one year old, 27% were one year old, 11% were 2 years old and 8% were 3 years old.

Five clusters involved 12 patients, 10 of *Salmonella newport* and two of *S. typhimurium*. Common foods were not a factor in these clusters.

Pulsed field gel electrophoresis of genomic DNA was studied for fingerprinting *Salmonella* isolates obtained from the Arkansas State Health Department. 22 isolates of *S. typhimurium* and 29 isolates of *S. newport* did not support identification of common sources but 17 isolates of *S. meunster* were identified to a common barbequed beef source.
Segregated Early Weaning -- Control of Food Safety Organisms

J. McKean*1, P. Fedorka-Cray2, I. Wesley2, J. Dickson1, P. Holden1, Elsa Murano1
1Iowa State University, Ames, Iowa and 2National Animal Disease Center, Ames, Iowa

Segregated early weaning (SEW) is a production technology gaining widespread acceptance in the North American swine industry to reduce or eliminate vertical transmission for common bacterial and viral swine pathogens. Food safety concerns have been raised about a variety of zoonotic bacterial species (Salmonella, Yersinia, Campylobacter, Arcobacter and Listeria) known to colonize normal swine or to contaminate pork products. (1) Because SEW technology functions to reduce swine pathogens it has been postulated that similar mechanisms could reduce important zoonotic bacteria at the production level. In this study we investigated the effect of SEW on the transmission of several zoonotic organisms in SEW swine and in conventional market swine. This study reports the first evaluation of SEW techniques to control zoonotic pathogens under commercial conditions.

Materials and Methods

Eight early weaned pigs from 61 commercial Iowa swine farms (487 pigs) were commingled at 16-18 days of age in two nursery buildings on a neutral site. Upon arrival, each pig was identified, weighed, rectal swabbed for zoonotic pathogens and a blood sample was drawn by jugular venipuncture. Pigs were allotted to pens by weight, were given a standard metaphylaxis program and were fed the same two fortified diets for the 49-day nursery period. Rectal swabs, obtained at 42 days post arrival, were examined for the presence of zoonotic pathogens. At 49 days post arrival all pigs were transferred to an off-site finishing location and regrouped into solid-partitioned, solid-floored, open-front pens by farm of origin. All pigs received the same grower and finisher diets until market weight of approximately 115 kg BW. Rectal swabs and a blood sample were obtained from all swine approximately 100 days post arrival to the finishing site. Rectal swab samples, obtained from 39 of the 61 cooperating farms and collected from a market cohort of the same age as the SEW pigs, were evaluated in the same manner. All pigs were marketed as they reached the 110 kg BW range. Individual pig weight gain and group feed efficiency in the nursery and individual weight gain and pen feed efficiency in the finishing phases were measured. Individual lesion scores for liver, lung, snout and skin using the PigMon reporting system(2) were recorded at slaughter. Carcass quality measurements were taken following the NPPC Genetic Evaluation Protocol.(3)

Results

Nursery performance for the 487 early weaned (16-18 days of age) pigs was within expected production parameters. Entry weights ranged from 3.75 to 8.4 kg, a mean 5.2 kg BW. Nursery exit weights ranged from 11 to 32.9 kg BW. Individual average daily gain ranged from 0.06 to 0.51 kg/day, a mean value of 0.34 kg/day. Entry weight did not significantly impact nursery exit weight except that the pigs with entry weight below 4.2 kg BW grew more slowly in both the nursery and finishing phases. Group nursery feed efficiency was 0.73 kg gain/kg of feed. Mortality during the nursery period was 2.54%, primarily from spontaneous coliform or S. suis septicemia. Finishing pigs performed at acceptable levels. The average beginning test weight was 21.3 kg and the average market weight was 110 kg. Average daily gain for all pens was 0.80 kg/day, but ranged from 0.64 to 0.95 kg/day. Feed efficiency ranged from 1.09 to 1.53 kg feed/kg gain.

Slaughter evaluations were conducted on individual pigs for health and carcass parameters. Lung, liver, skin, and snout lesions in the SEW pigs were minimal in prevalence and size. No significant differences in individual growth performance could be detected based on the presence/absence of liver, lung, or snout lesions in the SEW pigs. Carcass quality scores varied significantly based upon source herd. Average daily gain pen values ranged from a low of 0.23 to 0.38 kg lean/day, with an average value of 0.32 kg
lean/day. Efficiency of lean gain averaged 3.58 kg feed/kg of lean with a range from a low of 2.74 kg to a high of 4.3 kg feed/kg of gain.

The microbiologic evaluations demonstrated a wide range of prevalences for zoonotic pathogens within the populations. Individual pig results were aggregated to premises status for these analyses. A single positive culture denoted the premises as positive for that sampling period. The 61 farm culture results have been aggregated into SEW entry, 42 day, and 142 days post arrival and for the 39 of 61 farms represented in the finishing pigs on-farm columns of table 1.

On entry 16/487 (3.3%) pigs; at 42 days 1/484 (0.21%); and at 142 days post entry 1/470 (0.21%) pigs was *Salmonella* spp. culture positive. The nursery *Salmonella* spp. isolated were 13 *S. derby*, nine (9) *S. agona* and two (20) *S. anatum* serotypes. The only positive sample in the SEW finisher was a *S. choleraesuis* (kunzendorf) originating from a herd without prior positive culture results.

**Discussion**

The nursery and finishing performance parameters demonstrated that early weaned pigs from multiple sources of variable health status could be commingled with acceptable production results. The wide ranges in individual pig and pen performance was indicative of the substantial genetic range within these 61 herds. Pig weight at nursery entry, except for those below 4.2 kg BW, did not affect nursery or finisher growth performance. Similarly, no significant growth differences could be found between pigs with/without pathologic lesions. This observation is consistent with earlier evaluations of slaughter check data.

The microbiological collections were designed to build baseline data about the prevalence of zoonotic food safety pathogens in swine on commercial Iowa farms. A *Toxoplasma gondii* infestation rate of 11.4% (7/61 farms) at entry and in 14.75% (9/61 farms) at slaughter weight is consistent with preweaning exposure for individual pigs with no lateral spread. The two farms negative at entry may not have developed detectable antibodies prior to initial testing. The complete absence of *Yersinia* spp. and *Listeria* spp. from all animals was surprising. The decreasing prevalence for *Campylobacter*, *Arcobacter* and *Clostridium* spp. may indicate that pigs held under high health and hygiene conditions substantially reduce their carrier status with age. However, the on-farm *Campylobacter* controls at market weight were not different for prevalence when compared to SEW (41 vs 38%). Conversely, *Arcobacter* spp. prevalence was higher in SEW pigs than on-farm controls (84 vs 24%). *Salmonella* isolations decreased under high health conditions. The failure to isolate *Salmonella* from on-farm controls raises questions about whether the high health and hygiene environment represents a definitive control point of these zoonotic pathogens. Additional risk factors must be evaluated before effective and practical control methods for zoonotic microbial contaminants can be implemented at the production level.

**References**

Table 1. Sample positive herds for human food safety pathogens.

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Entry</th>
<th>SEW Pigs 42 D*</th>
<th>142 D</th>
<th>On-farm finisher pigs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Toxoplasma</td>
<td>7/61</td>
<td>ND</td>
<td>9/61</td>
<td>ND</td>
</tr>
<tr>
<td>Yersinia spp.</td>
<td>0/61</td>
<td>0/61</td>
<td>0/61</td>
<td>0/39</td>
</tr>
<tr>
<td>Listeria spp.</td>
<td>0/61</td>
<td>0/61</td>
<td>0/61</td>
<td>0/39</td>
</tr>
<tr>
<td>Campylobacter spp.</td>
<td>60/61</td>
<td>58/61</td>
<td>33/61</td>
<td>13/39</td>
</tr>
<tr>
<td>Arcobacter spp.</td>
<td>60/61</td>
<td>56/61</td>
<td>53/61</td>
<td>8/39</td>
</tr>
<tr>
<td>Clostridium spp.</td>
<td>61/61</td>
<td>61/61</td>
<td>3/61</td>
<td>ND</td>
</tr>
<tr>
<td>Salmonella spp.</td>
<td>8/61</td>
<td>5/61</td>
<td>1/61</td>
<td>0/39</td>
</tr>
</tbody>
</table>

*D = days

Table 2. *Salmonella* spp. isolations by farm.

<table>
<thead>
<tr>
<th>Farm #</th>
<th>Entry</th>
<th>SEW pigs 42 days</th>
<th>142 days</th>
<th>On-farm finisher pigs</th>
</tr>
</thead>
<tbody>
<tr>
<td>19</td>
<td>5/8 1 derby 4 agoana</td>
<td>8/8 negative</td>
<td>8/8 negative</td>
<td>ND</td>
</tr>
<tr>
<td>24</td>
<td>5/8 5 derby</td>
<td>8/8 negative</td>
<td>8/8 negative</td>
<td>negative</td>
</tr>
<tr>
<td>27</td>
<td>1/8 anatum</td>
<td>8/8 negative</td>
<td>8/8 negative</td>
<td>ND</td>
</tr>
<tr>
<td>29</td>
<td>2/8 2 derby</td>
<td>8/8 negative</td>
<td>8/8 negative</td>
<td>ND</td>
</tr>
<tr>
<td>33</td>
<td>1/8 1 derby</td>
<td>8/8 negative</td>
<td>8/8 negative</td>
<td>negative</td>
</tr>
<tr>
<td>36</td>
<td>8/8 negative</td>
<td>8/8 negative</td>
<td>1/8 choleraesuis (kunzendorf)</td>
<td>ND</td>
</tr>
<tr>
<td>45</td>
<td>1/8 1 derby</td>
<td>8/8 negative</td>
<td>8/8 negative</td>
<td>negative</td>
</tr>
<tr>
<td>59</td>
<td>1/8 1 derby</td>
<td>8/8 negative</td>
<td>8/8 negative</td>
<td>ND</td>
</tr>
<tr>
<td>60</td>
<td>8/8 negative</td>
<td>1/8 agoana</td>
<td>8/8 negative</td>
<td>negative</td>
</tr>
</tbody>
</table>
BREAKOUT GROUP REPORTS
Pathogenesis/Transmission Group Report

The pathogenesis/transmission breakout group identified 3 major questions that need to be addressed: 1) whether we are dealing with zero tolerance of *Salmonella* or whether some levels of *Salmonella* are acceptable, 2) what the transmission dynamics of *Salmonella* within herds is, 3) and what mechanisms lead to long-term colonization (possibly the development of carriers). It is assumed that overall responsibility for pathogen reduction will be a partnership between producers, packers, processors, and consumers.

The group concluded that the pre-harvest food safety objective should be directed toward pathogen reduction since it was felt that complete elimination of *Salmonella* with its ecologically diverse distribution is unlikely to be successful. It also was recommended that studies be performed to assess the current risk of food borne illness due to *Salmonella* as a result of consuming contaminated pork. Current estimates of prevalence should be used to make this assessment. Furthermore, a mathematical model should be developed that describes the effect that incremental reductions in risk has on the economic costs of disease. Factored in to this formula should be a measurement of the effect on the reduction of prevalence and reducing the number of infecting organisms per carcass. Ultimately this data will be used to set the total acceptable level of contamination permissible at the time of slaughter. One of the common themes of this question (and those to be described) is the need for common methods to detect (and quantify) *Salmonella* in pigs.

While many studies have been performed to determine the prevalence of *Salmonella* in swine, the epidemiology of *Salmonella*, particularly as it pertains to “carrier” animals and transmission through a herd, has not been well studied. The group recommends that studies be developed to understand the following:

1) What is a “carrier?”

2) Settle on standard methods and tissues for determining the presence of *Salmonella* in swine.

3) Perform systematic longitudinal studies of *Salmonella* in swine by necropsying animals of all ages during the on farm production cycle.

4) Determine the transmission dynamics/kinetics within herds and the sources of contamination.

5) Perform risk-assessment studies to identify factors affecting acquisition of *Salmonella* by swine.

6) Develop better and more cost-effective methods to detect *Salmonella*.

7) Determine host factors that contribute to sensitivity or resistance to *Salmonella* (e.g., genetics immunologic, or stresses/transportation).

8) Determine the relative importance of route of acquisition on *Salmonella* transmission and development of carriers.

The breakout group on Pathogenesis and Transmission felt that understanding the mechanism(s) of long-term colonization/carrier and the “virulence” factors associated with this process would likely lead to identifying targets to develop intervention procedures. It was recommended that this be a research priority. In addition, it
was recommended that:

1) The role of antibiotics on the establishment of “carriers” or long term colonization be assessed.

2) Studies should be performed to determine if vaccination with attenuated *Salmonella* vaccines reduces the risk of long-term colonization.

3) Studies should be performed to determine if animals can become super-infected with 2 or more serotypes of *Salmonella*.

4) With exception of host adapted serotypes, is contamination of swine with different serotypes of *Salmonella* likely to be more or less related to the risk of food borne illness in humans.

5) Do different serotypes require different intervention schemes.

6) Can competitive exclusion and selective enrichment of specific bacteria be used to effectively control *Salmonella* acquisition and transmission in a herd.
Feed Group Report

On Tuesday afternoon a general discussion of feed issues was held by the Group. The main work of the Group was conducted on Wednesday morning. The approach used was to address each question.

Question 1

The ongoing research will provide information on the role of transportation as a source of Salmonella contamination of feedstuffs. The importance of sanitizing/cleaning trucks was demonstrated at this meeting as was the importance of handling feed in a manner that will not lead to microbial contamination. The Group consensus was that as efforts are focused on improving management practices, feed will become a more important vector for Salmonella contamination of the animal. The Swedish experience has demonstrated this. The importance of classifying herds was emphasized and recommended. The Danish and Swedish experience show that it is important to focus efforts on herds with the most problems.

Question 2

The culture method is the current "gold standard" for determining whether Salmonella is present. Two culture methods are most common. The FDA BAM method and the USDA method used by Bailey-Cox and NVSL. The importance of pre-enrichment media and of using at least two selective media was emphasized. The current rapid detection methods using ELISA, DNA/RNA, PCR methodology all require pre-enrichment and thus, require 18 to 24 hours or more to complete. In addition, the sensitivity and specificity of these rapid methods in a feed matrix or with animal pathogens has not been well established since most were developed based on human clinical disease or for human food products. In addition since the rapid tests measure bacterial antigens or nucleic acids they do not differentiate well between viable and non-viable organism, nor between whole organism and "parts" of organisms. The viable/non-viable issue is both a regulatory problem as well as a problem with interpreting the significance of the results regarding the potential safety of the product. Finally, since several different methods and sampling plans are being used, comparing results is difficult. The Group recommended that a standard method and sampling scheme be established and used in future research.

Question 3

No new research in the feed area is being planned for next year. Several studies will be completed and the data made available. AOAC is preparing reference material and a validation scheme for laboratories working in this area. FDA will supply the organizers a summary of research it funded on evaluating culture methods and rapid detection methods in feed for inclusion in the Proceedings.

Question 4

Present knowledge on the role of feed as a vector for Salmonella in swine needs to have a wider distribution. The Group suggested getting the message to the producer through groups such as NPPC, AASP, and extension agents. Convincing producers to incorporate new management and feeding practices will likely require that research be able to demonstrate both an improvement in the level of safety associated with the product and an economic advantage.
Question 5

Research needs to demonstrate the management benefits of QA programs and the benefits of 
Salmonella negative feed. Research in methodology needs to focus on a rapid "truck-side" test for 
detecting Salmonella. Current tests require 24 to 48 hours and are thus not useful for the feed 
industry because of the manner in which feed is manufactured and sold. If a few serotypes or 
serogroups could be identified as important in feed, then constructing a rapid test might be easier. 
In order not to miss an emerging serotype, routine surveillance would still use methods that 
detected all serotypes.

Question 6

NAHMS is gathering data on Salmonella in feeds in the farm environment and will share this data. 
NAHMS will rely on those in Salmonella research to provide expert interpretation of the data. 
KSU has facilities and the ability to conduct animal trials. FPRF and APPI have both funding and 
experience with controlling Salmonella in feed ingredients and are willing to collaborate / cooperate 
in feed studies. FDA / CVM will share data on Salmonella methodology for use in feeds.

Question 7

Addressed in above responses.
HACCP Group Report

Goals established for the HACCP breakout

1. It was considered premature to discuss HACCP in formal terms because of the lack of information necessary to identify potential critical control points on farms.

2. Goal to identify key areas in the production chain where information is required.

3. Describe what knowledge we have / don't have in each important area and define priorities for research.

Assumptions and outline of discussion

Our basic assumptions were that reducing Salmonella at the farm level will reduce Salmonella in pork, and that the ultimate goal is less Salmonella on pork presented to consumers. A simple flow chart of the production process was used to develop discussions. The process extended from inputs to breeding herds through to transport of finished pigs. Discussion was limited to the production process and did not include slaughter and processing. Slaughter was excluded owing to time constraints, inadequate experience of the group with slaughter processing, and the fact that no information on slaughter aspects had been presented at the symposium.

Factors important throughout the production process

1. Environment and management - undoubtedly important and poorly understood. Further research of the role of management and environmental factors is necessary.

2. Waste handling - as above.

3. Rodent control - research in poultry and swine indicates rodents are important reservoirs of Salmonella. Further research of the role of rodents is not a high priority, but rodent control is important on farms.

4. Transportation, both of animals and other inputs, is potentially important. Available evidence suggests transport results in increased shedding of Salmonella in pigs sent for slaughter. The role of transport between stages of production, depending on production system, needs to be investigated. The mechanism of the apparent affect of transportation is not known (expression of existing carrier state, truck hygiene, mixing pigs, transfer to a contaminated environment with rapid colonization).

General issues were identified that apply to multiple levels, and need to be standardized or have general consensus as to approach:

1. sampling objectives and collection of samples

2. testing methodologies (also see sampling notes at the end of the discussion)
   i. Bacteriological methods
   ii. Serological methods
   iii. Survey questions and measurement of putative risk factors
Breeding herd

The following inputs for the breeding herd were discussed

1. Feed - important; further research low priority
2. Breeding stock - potentially important. No information in U.S.
3. Water - unknown
4. Other (e.g. labor)

Some information presented suggests that segregated early weaning may be effective in preventing sow-to-piglet transmission. However, there is little information about the importance of the sow as a source of infection of growing pigs. Further work is needed to evaluate the role of the sow as a source of infection of piglets and of the efficacy of early weaning protocols.

Nursery

No data on prevalence in nursery pigs in different systems. Impact of nursery environment on Salmonella status of finishing pigs is not known. Evaluation of prevalence and the role of nursery infection as a determinant of finishing prevalence is required before evaluation of risk factors associated with nursery management. Longitudinal studies would be preferable, although serial cross-sectional studies may also be adequate. The role of other diseases in nursery pigs is also unknown.

Finishing

Further information on prevalence in finishing pigs is not a high priority. NAHMS data and data from several other studies have indicated that Salmonella are prevalent in many finishing operations. Future research should focus on risk factors for prevalence of infection in finishing populations and the relative importance of sources of Salmonella.

Summary - key areas for future research focus:

1. Sow to pig transmission
   a. significance of sows status in transmission, within production type and management systems.
   b. weaning techniques

2. Transportation
   a. Mechanisms - stress (expression of existing, undetected infection) vs. physical contamination and/or infection during transport and lairage
   b. Three transportation areas
      i. inputs (feed, etc.)
      ii. within production system transportation (such as between stages)
      iii. delivery to market

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3. Nursery
   a. prevalence needs to be defined
   b. epidemiology if nursery infection found to be important

4. Finishing
   a. Prevalence does not need to be better defined in the national herd; enough information is available in existing and developing studies (assuming that the detection methodology has been adequate, see general issues above.)
   b. Specific risk factors need to be determined.
      i. Standardized evaluation of risk factors among studies would be useful.
      ii. Flooring
      iii. Air quality
      iv. Rodents
      v. Density - regional (GIS) and within farms

5. General Methodology - it was felt that standardization of methods among investigators would be beneficial to interpreting results from different studies. Standardization should be considered with respect to bacteriological methods, sampling approach (sample size, selection of pigs, selection of tissues, location of collection (i.e., at farm gate, at lairage, etc.). A proposal from the group is that at least 2 working groups could be formed to evaluate the potential for standardizing experimental methods among studies. One group would focus on standardization of microbiological methods (including tissue collection, culture and serology). A second group would focus on design of risk factor studies (i.e. standardization of survey questions, methods for measuring risk factors on farms, etc).
FDA’s Role in Feed Safety

Daniel G. McChesney, PhD

In discussing FDA’s role in feed safety I will concentrate on three areas: 1) FDA’s authority for jurisdiction over feeds, 2) the application of HACCP to the feed industry, and 3) the results of FDA’s recent survey on the prevalence of Salmonella contamination of complete feeds and the primary meal ingredient.

The Food and Drug Administration (FDA) has primary responsibility in the federal government for food safety in the United States. FDA is charged with the enforcement of the Federal Food, Drug, and Cosmetic Act (FFDCA), and food safety related aspects of the Public Health Service Act (PHSA). Under these Acts, a part of the FDA responsibility is to ensure that human foods and animal feeds are safe and, among other things, do not contain illegal residues of drugs, pesticides, environmental contaminants, or microorganisms, including mycotoxins and bacterial toxins, that are harmful to public health. The Department of Agriculture (USDA) has responsibility for the safety of human food products resulting from the slaughter of food animals under the Meat and Poultry Inspection Act.

The FDA mandate under the PHSA and the FFDCA includes widespread responsibilities to help ensure preharvest food safety. For example, one mission of FDA’s Center for Veterinary Medicine (CVM) is to regulate the levels of contaminants permitted in animal feeds to ensure that the food for man and animals is safe and free of illegal drugs, industrial chemical, pesticide residues, and harmful bacteria.

The FFDCA defines food as "articles used for food or drink for man or other animals . . . and articles used for components of any such article." Therefore, any product that is intended to be used as a feed ingredient or become part of an ingredient or feed is considered a “food” under the FFDCA and thus subject to regulation. Furthermore, it is the position of FDA that a product intended for use as a feed or feed ingredient must not be adulterated as defined in Section 402 (a) of the Federal Food, Drug, and Cosmetic Act. Section 402 (a) of the Act has numerous provisions for establishing adulteration. The most appropriate subsections of 402 to apply are (a) (1) and (a) (3). Section 402 (a) (1) states in part that a food (feed) shall be deemed to be adulterated “if it bears or contains any poisonous or deleterious substance which may render it injurious to health” and subsection (a) (3) states in part “a food shall be deemed to be adulterated if it is otherwise unfit for food (feed).” Additionally, Section 402 (a) (2) (C) states that a food (feed or feed ingredient) can be considered adulterated if “it bears or contains any food additive which is unsafe (unapproved) within the meaning of Section 409.”
HACCP

CVM has been emphasizing HACCP for over 2 years as an approach for achieving Salmonella negative feed. Because of this, major segments of the feed industry have developed a basic understanding of HACCP and how it might be applied to a particular segment of the feed industry.

FDA is focusing on a HACCP approach because it is preventative, addresses the root causes of food safety; problems in production, storage, transportation, etc., is applicable to both human food and animal feed, and industry plays a central role in the development of the program. The two principle alternatives, increased end-product testing and comprehensive GMPs, lack the distinct advantage of a HACCP-based approach.

GMPs have and continue to work effectively for specific areas in both human food and animal feed. Examples of successful application of GMPs are the food sanitary GMPs, outlined in 21 CFR, Part 110, and the medicated feed GMPs outlined in 21 CFR, Part 225. In both these instances, GMPs address specific problems and control points that are specific and common to all members of the industries to which they are applied. Because of the specific nature of GMPs, they are not particularly well suited to operations within an industry with great diversity such as the human food industry or the non-medicated animal feed industry. Thus, while sanitary GMPs can be equally applied to the manufacture of cheese or green beans, the GMPs for cheese making and green bean processing would be very different. Likewise, sanitary GMPs could be applied to a rendering plant and a feedmill but the GMPs for each process would be very different. Therefore, GMPs for the food and feed industry, while possible, are not practical because of the breadth and diversity industries, and the resources that would be required to cover the industries.

Before beginning a discussion of HACCP programs for feed, I would like to address quality assurance programs that are already in place. Some of these may not meet the whole definition of HACCP but parts of them may satisfy some requirements of the overall HACCP program. If a processor has a quality assurance program in place that enables the production of a safe product through process controls then we would suggest that the processor evaluate its program, and determine how to adjust it to meet the HACCP approach. It is not FDA’s intention that firms scrap functioning quality assurance programs but rather that they review and expand them in order to improve the programs.

As FDA gains experience with HACCP and industry begins developing HACCP programs, it is becoming clear that there is confusion over critical control points. In most cases, industry has identified additional critical control points in areas that are either not directly related to the safety of the product or have included critical control points for economic issues, equipment safety, and sanitation. In some cases, this stems from not understanding that critical control points are meant to deal with safety issues and should occur at points in the process where the safety hazard can be reduced or eliminated. In other cases, it has resulted from not clearly identifying the hazard. The approach FDA is using in evaluating HACCP programs for the Pilot Plant Program for human food incorporates the concept of prerequisite programs. From the plants in the Pilot Program it has become clear that controlling process steps for which a safety hazard has either not been identified or the step cannot eliminate or reduce the safety hazard is better addressed through prerequisite programs (i.e. sanitary GMPs, SOPs, etc.) then as critical control points within the HACCP program. When the prerequisite program approach is combined with a HACCP program, the number of critical control points is greatly reduced and the entire process becomes much more manageable.

An example of an area in a feedmill in which a prerequisite program may be more appropriate is
REVIEWS
sanitation. Inadequate sanitation is an ongoing problem that needs to be addressed for both personnel and facilities. However, from a sanitation point of view, every part on the plant has the potential to be a critical control point. Identifying sanitation critical control points and including them in the HACCP plan can result in the HACCP plan becoming so unfocused as to be meaningless. Because of this, we suggest that sanitary guidelines be developed and incorporated into a prerequisite program rather than the HACCP plan. Standard operating procedures for sanitation should address personal hygiene; maintenance of building, facilities, and grounds; sanitary facilities and controls; production and process controls; and training. Sanitary guidelines often include sanitizing and maintenance of equipment and utensils, however, in some instances it may be appropriate to include these last two items in the HACCP plan.

Other examples of where prerequisite programs may be a more appropriate manner of handling a potential problem are the receiving of incoming material and the post process handling of the finished product. The decision on the use of a prerequisite program or a critical control point to control a potential problem is based on the hazard involved, the ability of the step/process to control or eliminate the hazard, and whether another step in the process will remove or reduce the hazard.

We are very encouraged by the feed industry's response in the last year to implementing the HACCP approach. The rendering industry, through APPI, and the fishmeal industry under the National Marine Fisheries Service, are actively developing HACCP plans for use by participating firms. The National Grain and Feed Association (NGFA) and the American Feed Industry Association (AFIA) have published quality assurance programs for their members that are developed around existing cGMPs and incorporate many aspects of a HACCP plan. AFIA has recently published a pamphlet entitled “HACCP-Type Guidelines for Feed Manufacturing Facility Salmonella Control.” In addition, several producer organizations are working on HACCP plans for their segments of animal agriculture.
Survey

Purpose:

FDA district offices are collecting samples of complete feed and the primary animal or vegetable meal in the feed. The purpose of the survey is to collect information on the prevalence of salmonella in these products. We will use the information to establish a baseline.

Methods:

Seventy-five commercial and twenty-five non-commercial feedmills were selected for inclusion in the survey. The on-farm mixers were randomly selected from FDA’s Official Establishment Inventory (OEI). Each district was assigned a specific number of commercial feed mills based on the number of registered feed mills within the district. The selection of the feed mills at which samples were collected was determined by the district. However, the district was instructed to select mills that would be representative of the feeds manufactured in the district. Medicated feeds were included in the sampling. The results I am reporting today represent data from 1,980 meal subsamples and 1,860 complete feed subsamples covering 66 meals and 62 complete feeds, respectively.

Each sample of meal and complete feed consisted of 30 individual subsamples (66 x 30 = 1,980; 62 x 30 = 1,860) of approximately 100 grams, each of which were aseptically collected. The 30 subsamples were analyzed as two composite samples (subsamples 1-15 and 16-30) using the procedure for Salmonella isolation and identification stated in the 7th edition of the Bacteriological Analytical Manual (BAM) and included serogrouping and serotyping.

This sampling plan has the theoretical possibility of detecting 1 organism in 278 grams of product. Another way of stating this is that if 5% of the sample units in a lot contain Salmonella there is a 21% chance that the sampling plan will not detect Salmonella and thus, incorrectly report (false negative) that the lot is Salmonella negative. However, if 10% of the sample units in a lot contain Salmonella there is only a 4% chance that the sampling plan will not detect Salmonella, and at 20%, less than a 1% chance that Salmonella will not be detected. This sampling plan was chosen by FDA to begin establishing a baseline of Salmonella prevalence data. If a less stringent sampling plan was used it would have been difficult to detect Salmonella at low prevalence levels. On a positive note, the stringency of the sampling plan means that all plants testing negative, had, on that day and for the specific product, a greater than a 99% chance of having product that had less than 20% of the analytical units positive for Salmonella.

(Data presentation)

TABLES ATTACHED

Data Summary:

Sixteen percent of the complete feeds and 48% of the meals were positive for Salmonella. When the meals were grouped by animal and vegetable source, 82% of the animal meals and 37% of the vegetable meals were positive for Salmonella. When the meal and complete feed pairs were compared, if the meal was positive, the complete feed was also positive in 30% of the samples. Alternatively, 70% of feed samples made with positive meal were negative for Salmonella. There was one case in which the meal was negative and the complete feed positive (3%) and 32 cases in which both the meal and feed were negative (97%).
Discussion:

As in the previous study of protein meals, animal protein meal was contaminated with Salmonella more often than vegetable protein meal. However, the idea that simply eliminating animal protein from rations as the sole means of effectively preventing the introduction of Salmonella in feed is still a flawed strategy. Producing a complete feed using meal ingredients which are negative for Salmonella, appears to be an effective strategy for minimizing the possibility of producing a Salmonella positive complete feed, provided the process control steps that are currently in place remain.

Enforcement:

CVM will continue to support regulatory action recommended by the Field against feed, ingredients, or pet food presenting a hazard to human or animal health. While we are concerned with all reports of Salmonella contamination in animal feed, we are most concerned with Salmonella contamination of retail size containers which can be expected to be taken into the home where contamination might extend to cooking utensils and food preparation surfaces, thereby exposing humans to the contaminant.

This concludes my presentation. Thank you.
POWER OF SAMPLING PLAN

<table>
<thead>
<tr>
<th>C</th>
<th>P</th>
<th>N = 10</th>
<th>N = 30</th>
</tr>
</thead>
<tbody>
<tr>
<td>F</td>
<td>F</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>60</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>35</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>20</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>11</td>
<td>&lt;1</td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>35</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>&lt;1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

N = NUMBER  
P = PREVALENCE  
C = ACCEPT CRITERIA (0 = NO POSITIVES ALLOWED)  
F = % FALSE NEGATIVE
TABLE I

**SALMONELLA SURVEY RESULTS OF COMPLETE FEED AND THE PRINCIPLE PROTEIN MEAL**

<table>
<thead>
<tr>
<th></th>
<th>POSITIVE SAMPLES</th>
<th>TOTAL SAMPLES</th>
<th>PERCENT POSITIVE</th>
</tr>
</thead>
<tbody>
<tr>
<td>FEED</td>
<td>10</td>
<td>62</td>
<td>16.1</td>
</tr>
<tr>
<td>MEAL</td>
<td>32</td>
<td>66</td>
<td>48.5</td>
</tr>
<tr>
<td>TOTAL</td>
<td>42</td>
<td>128</td>
<td>32.8</td>
</tr>
</tbody>
</table>

1. Contains miscellaneous feed
2. Reports pending for 4 feed and 3 meal samples

TABLE II

**SALMONELLA RESULTS FOR ANIMAL AND VEGETABLE PROTEIN MEAL**

<table>
<thead>
<tr>
<th>MEAL</th>
<th>POSITIVE SAMPLES</th>
<th>TOTAL SAMPLES</th>
<th>% POSITIVE SAMPLES</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANIMAL</td>
<td>14</td>
<td>17</td>
<td>82.4&lt;sup&gt;1&lt;/sup&gt;</td>
</tr>
<tr>
<td>VEGETABLE</td>
<td>18</td>
<td>49</td>
<td>36.7&lt;sup&gt;1&lt;/sup&gt;</td>
</tr>
<tr>
<td>TOTAL</td>
<td>32</td>
<td>66</td>
<td></td>
</tr>
</tbody>
</table>

1. % Positive plants are significantly different at p < 0.01
TABLE III

SAMPLE RESULTS BY FEED TYPE

<table>
<thead>
<tr>
<th>FEED</th>
<th>POSITIVE SAMPLES</th>
<th>TOTAL SAMPLES</th>
<th>PERCENT POSITIVE</th>
</tr>
</thead>
<tbody>
<tr>
<td>CATTLE</td>
<td>1</td>
<td>7</td>
<td>14.3</td>
</tr>
<tr>
<td>DAIRY</td>
<td>2</td>
<td>7</td>
<td>28.6</td>
</tr>
<tr>
<td>MISC.</td>
<td>0</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>POULTRY</td>
<td>4</td>
<td>27</td>
<td>14.8</td>
</tr>
<tr>
<td>SHEEP</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>SWINE</td>
<td>3</td>
<td>15</td>
<td>20</td>
</tr>
<tr>
<td>TOTAL</td>
<td>10</td>
<td>62</td>
<td>16.1</td>
</tr>
</tbody>
</table>

1. 12 of 27 of the poultry feed samples were obtained from “Growers.”

Of the 4 positive poultry feeds, 3 used positive meat and bone meal and 1 used positive soybean meal. Seven additional feeds were made with a positive meal (1 meat and bone meal, 1 meat meal, 1 poultry meal, 3 soybean meal, and 1 sunflower meal). None of the additional seven feeds made from positive meal were positive for Salmonella. 6 of the 27 feeds contained positive animal protein meal and 5 of 27 feeds contained positive vegetable meal. Three of the positive feeds were from commercial feedmills and one from a Grower. The Grower’s feed contained positive meat and bone meal.

There were 7 medicated poultry feed samples, all were negative. One medicated feed was made with positive soybean meal. Three medicated feeds were from Growers and 4 from commercial mills. Two feeds did not have a meal pair.
### TABLE IV

**ANIMAL PROTEIN MEAL SAMPLES**

<table>
<thead>
<tr>
<th>TYPE MEAL</th>
<th>POSITIVE SAMPLES</th>
<th>TOTAL SAMPLES</th>
</tr>
</thead>
<tbody>
<tr>
<td>EGG SPRAY DRIED</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>FISH</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>MEAT/BONE MEAL</td>
<td>8</td>
<td>9</td>
</tr>
<tr>
<td>MEAT MEAL</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>POULTRY MEAL</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td><strong>SUBTOTAL</strong></td>
<td><strong>14</strong></td>
<td><strong>17</strong></td>
</tr>
</tbody>
</table>

### TABLE V

**VEGETABLE PROTEIN MEAL SAMPLES**

<table>
<thead>
<tr>
<th>TYPE MEAL</th>
<th>POSITIVE SAMPLES</th>
<th>TOTAL SAMPLES</th>
</tr>
</thead>
<tbody>
<tr>
<td>CANOLA MEAL</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>CORN MEAL</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>CORN GLUTEN MEAL</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>COTTONSEED MEAL</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>SOYBEAN MEAL</td>
<td>14</td>
<td>36</td>
</tr>
<tr>
<td>SUNFLOWER MEAL</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>WHEAT MILL RUN</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td><strong>SUBTOTAL</strong></td>
<td><strong>18</strong></td>
<td><strong>49</strong></td>
</tr>
<tr>
<td><strong>GRAND TOTAL</strong></td>
<td><strong>32</strong></td>
<td><strong>66</strong></td>
</tr>
</tbody>
</table>
TABLE VI

INFLUENCE OF *SALMONELLA* STATUS OF PROTEIN MEAL ON *SALMONELLA* STATUS OF COMPLETE FEED

*SALMONELLA* STATUS

<table>
<thead>
<tr>
<th>MEAL</th>
<th>FEED</th>
<th># OF PAIRS</th>
<th>% OF PAIRS</th>
</tr>
</thead>
<tbody>
<tr>
<td>POSITIVE</td>
<td>POSITIVE</td>
<td>8</td>
<td>29.6</td>
</tr>
<tr>
<td>POSITIVE</td>
<td>NEGATIVE</td>
<td>19</td>
<td>70.4</td>
</tr>
<tr>
<td>SUBTOTAL</td>
<td></td>
<td>27</td>
<td></td>
</tr>
<tr>
<td>NEGATIVE</td>
<td>POSITIVE</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>NEGATIVE</td>
<td>NEGATIVE</td>
<td>32</td>
<td>97</td>
</tr>
<tr>
<td>SUBTOTAL</td>
<td></td>
<td>33</td>
<td></td>
</tr>
<tr>
<td>TOTAL</td>
<td></td>
<td>601</td>
<td></td>
</tr>
</tbody>
</table>

1. There were 5 positive meals without a feed pair, 1 positive feed without a meal pair.
### TABLE III (swine)

**SAMPLE RESULTS BY FEED TYPE**

<table>
<thead>
<tr>
<th>FEED</th>
<th>POSITIVE SAMPLES</th>
<th>TOTAL SAMPLES</th>
<th>PERCENT POSITIVE</th>
</tr>
</thead>
<tbody>
<tr>
<td>CATTLE</td>
<td>1</td>
<td>7</td>
<td>14.3</td>
</tr>
<tr>
<td>DAIRY</td>
<td>2</td>
<td>7</td>
<td>28.6</td>
</tr>
<tr>
<td>MISC.</td>
<td>0</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>POULTRY</td>
<td>4</td>
<td>27</td>
<td>14.8</td>
</tr>
<tr>
<td>SHEEP</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>SWINE(^1)</td>
<td>3</td>
<td>15</td>
<td>20</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>10</strong></td>
<td><strong>62</strong></td>
<td><strong>16.1</strong></td>
</tr>
</tbody>
</table>

1. 2 of 13 of the swine feed samples were obtained from “Growers.”

Of the 3 positive swine feeds, 2 used positive meat and bone meal and 1 used positive meat meal. Six additional feeds were made with a positive meal (4 soybean meal, 1 meat meal, and 1 meat and bone meal). None of the additional six feeds made from positive meal were positive for *Salmonella*. 5 of the 15 feeds contained positive animal protein meal and 4 of 15 feeds contained positive vegetable meal. All of the positive feeds were from commercial feedmills.

There were 2 medicated swine feed samples, both were negative. The three pelleted feeds and one mash feed were negative.
TABLE III (dairy and cattle)

SAMPLE RESULTS BY FEED TYPE

<table>
<thead>
<tr>
<th>FEED</th>
<th>POSITIVE SAMPLES</th>
<th>TOTAL SAMPLES</th>
<th>PERCENT POSITIVE</th>
</tr>
</thead>
<tbody>
<tr>
<td>CATTLE¹</td>
<td>1</td>
<td>7</td>
<td>14.3</td>
</tr>
<tr>
<td>DAIRY¹</td>
<td>2</td>
<td>7</td>
<td>28.6</td>
</tr>
<tr>
<td>MISC.</td>
<td>0</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>POULTRY</td>
<td>4</td>
<td>27</td>
<td>14.8</td>
</tr>
<tr>
<td>SHEEP</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>SWINE</td>
<td>3</td>
<td>15</td>
<td>20</td>
</tr>
<tr>
<td>TOTAL</td>
<td>10</td>
<td>62</td>
<td>16.1</td>
</tr>
</tbody>
</table>

1. 4 of 7 of the dairy feed samples were obtained from “Growers.” None of the cattle feed samples were obtained from “Growers.”

Of the 2 positive dairy feeds, 1 used positive cottonseed meal and the other used negative soybean meal. The one positive cattle feed did not have a meal pair. One additional dairy feed and 3 additional cattle feeds were made with positive meals (cottonseed meal (d), and one each of cottonseed meal, soybean meal, and sunflower meal, for cattle feed. None of the additional four feeds made from positive meal were positive for *Salmonella*. None of the dairy or cattle feeds contained animal protein meals as the primary meal. The 3 positive feeds were from commercial feedmills.

There was 1 medicated cattle feed sample and it was negative. There were no medicated dairy feeds.
SALMONELLOSIS IN SWINE; A REVIEW OF SIGNIFICANT AREAS AFFECTING THE CARRIER STATE.
Jeffrey T. Gray, PhD and Paula J. Fedorka-Cray, PhD

THE ORGANISM

Introduction

Salmonella species (spp.) are facultatively anaerobic, non-spore forming, Gram-negative, facultative intracellular bacteria which belong to the family Enterobacteriaceae. The majority of Salmonella are motile, however, nonmotile mutants may occur and one serotype, S. pullorum is always nonmotile (LeMinor 1984). Infection of animals with various species of Salmonella may or may not result in serious disease. It does, however, serve as a reservoir for potential transmission to humans. The interplay of Salmonella spp. with its host is varied and may include host specificity, inapparent infections, recovered carriers (subclinical carriers), enteritis, septicemia, abortion and combinations of disease syndromes. Salmonella spp. are zoonotic agents and are readily transferred between animals, from animals to humans and between humans by direct or indirect means.

Classification

There is a variety of naming schemes associated with the genus Salmonella none of which are completely accepted by scientists in the field. DNA-DNA hybridization between salmonellas has indicated that there is not enough genetic variation to warrant species differentiation within the Salmonella genus (Hook 1990). Another study has divided the genus into three species, S. typhi, S. choleraesuis, and S. enteritidis (LeMinor 1984). In this schema S. typhi and S. choleraesuis each consist of a single serotype, while all other Salmonella are grouped under S. enteritidis.

Currently, it is recognized that the genus Salmonella is divided into two species, S. enterica and S. bongori. Salmonella enterica is further subdivided into six subspecies: enterica, salmiae, arizonae, diarizonae, indica and houtenae (Leminen and Popoff 1987; Reeves et al. 1989). Most Salmonella belong to S. enterica subsp. enterica. Members of this subspecies are given a name which is usually based on the geographic location where the serovar was first isolated.

At the present time, there are approximately 2300 serotypes of Salmonella which differ in antigenic structure, host adaptation and biochemical reactions. The most widely used, and probably the most useful method of differentiating between Salmonella spp., is the Kauffmann-White scheme (Hook 1990). Serotypes are differentiated by exhaustive cross-absorption and cross-reaction with antisera from the existing serotypes. The antigens responsible for differentiation are the somatic O antigens, as well as the flagellar H antigens and the Vi antigen (Falkow and Mekalanos 1990). Using this system Salmonella are named using a genus species format such as S. typhimurium. However, the species nomenclature clearly does not define a species but rather a serotype of Salmonella.

PATHOGENESIS

Infectious dose

While ingestion of large numbers of Salmonella spp. may be required to initiate disease, disease is facilitated often by factors such as peristaltic impairment, interference with intestinal flora and elevation of gastric pH (Clarke and Gyles 1993). The LD<sub>50</sub> for S. enteritidis in germ free mice has been shown to be only 3-5 organisms. However, the comparable value in conventional mice is 10<sup>6</sup> CFU (Collins and Carter 1978). These data have implicated normal intestinal flora as one protective factor from development of clinical salmonellosis. They may also serve to explain the greater susceptibility of the very young whose intestinal flora is not fully developed.

Most studies suggest Salmonella spp. gain access to the host by an oral route, pass through
the stomach (during which time populations are greatly reduced), then colonize the intestine (Hale 1988). Intraluminal replication varies between serotypes. In swine, *S. typhimurium* replicates to much higher numbers intraluminally than *S. choleraesuis* which is inherently more invasive (Gianella et al. 1973; Reed et al. 1986).

**Adherence**

Attachment of *Salmonella* spp. to epithelial cells has been shown to be influenced by a nonfimbrial, mannose-resistant adhesin which can mediate attachment of *Salmonella* spp. to mammalian tissue culture cells in vitro (Tavendale et al. 1983). Interestingly, many of the serotypes which are less invasive in humans have the mannose-resistant adhesin, whereas the more invasive ones, such as *S. choleraesuis* and *S. typhi* lack the adhesin (Clarke and Gyles 1993). This indicates that other virulence factors mediate disease outcome. Most serotypes of *Salmonella* possess mannose-sensitive hemagglutinating pili (type 1) that bind to mannose derivatives on eukaryotic cells. However, the type 1 pili do not appear to play a significant role in adherence of the bacterium to the ileal mucosa (Finlay and Falkow 1988).

**Invasion**

Much of the knowledge regarding the penetration of the intestine by *Salmonella* spp. is based on work by Takeuchi (1967). The bacteria enter enterocytes through the microvilli or via the tight junctions in between enterocytes then migrate via membrane-bound vesicles to the basal region of the cell. The *Salmonella* pass through the enterocytes to the lamina propria where they stimulate an inflammatory response and are phagocytized by neutrophils and macrophages (Takeuchi and Sprinz 1967).

More recent studies have looked at the invasion process on a molecular basis and have divided this process into two stages. In the first stage, the bacteria adhere to an unidentified receptor on the epithelial cell surface and cause activation of a cascade of events on the cell surface which are mediated by the epidermal growth factor receptor (Galan et al. 1992b; Portnoy and Smith 1992). This series of events indicates that *Salmonella* invasion is dependent on both virulence factors of the pathogen and on the interaction of the host cell with the pathogen. In the second stage, invasion by *Salmonella* spp. induces a tyrosine phosphorylation of the epidermal growth factor receptor. It appears this phosphorylation induces increased intracellular calcium concentrations, microvilli depolarization, formation of extracellular blebs and internalization of the organism (Galan et al. 1992a; Portnoy and Smith 1992). It has been shown that invasion is regulated by several global regulatory systems which are induced by environmental factors such as low oxygen concentration, temperature, and osmolality (Galan and Curtiss 1990; Lee and Falkow 1990; Jones et al. 1992). Other factors influencing invasion include chemotaxis, motility and flagellar orientation (Jones et al. 1992). However, plasmids, which have been shown to be critical for virulence, have no effect on invasion (Gulig and Curtiss 1987).

**Intracellular survival**

Once inside the cell, *Salmonella* spp. multiply in membrane-limited vacuoles with a generation time of about 50 minutes. *Salmonella* spp. can survive within many cells; however, the most significant cell is probably the macrophage. Mutations which destroy this capacity include loss of LPS and certain auxotrophic and regulatory events. Cell wall composition has been shown to influence intracellular survival of *S. choleraesuis* (Griffith 1982). In the intracellular compartment the bacteria are protected from nonspecific defenses such as complement as well as antibody and some antibiotics (Falkow and Mekalanos 1990).

*Salmonella* spp. synthesize over 30 proteins which are selectively induced during infection of macrophages. Two of these are heat shock proteins, GroEL and DnaK. Avirulent, macrophage sensitive mutants have been shown to produce heat shock proteins but fail to synthesize different subsets of proteins normally induced within the macrophage. This indicates that these proteins are important for survival (Buchmeier and Heffron 1990).
Intestinal inflammation, fluid production

Salmonella spp. are known to produce cholera-like and shiga-like enterotoxins and these toxins may induce diarrhea independent of mucosal damage (Kinsey et al. 1976; Clarke and Gyles 1987). The Salmonella enterotoxin gene has been shown to be coded on the chromosome (Chopra et al. 1987). It must be noted however, that the production of toxins in vitro is somewhat obscure and not easily studied in the case of Salmonella. Therefore, the overall importance of toxin production is not understood. In general, diarrhea observed with salmonellosis is believed to be primarily associated with the inflammatory response induced by Salmonella spp. (Gianella 1979). This response stimulates local prostaglandin synthesis resulting in activation of the adenylate cyclase system increasing the secretion of fluid and electrolytes into the lumen (Falkow and Mekalanos 1990). The systemic signs and lesions relating to the septic form of this disease are commonly attributed to endotoxemia as a result of bacterial dissemination.

Cell free extracts of Salmonella have been shown to be cytotoxic and inhibit protein synthesis in eukaryotic cells (Koo and Peterson 1982, Koo et al. 1984). These studies provide a molecular basis for the cellular damage caused by Salmonella cytotoxin during experimental salmonellosis (Koo et al. 1984).

Extraintestinal infection

The struggle between Salmonella spp. and the host is usually not localized in the intestine. This is especially true with S. choleraesuis infection (Cherubin et al. 1974; Wilcock 1979; Reed et al. 1986). Bacteria which may be intracellular or free in the mucosa and submucosa are transported by the lymphatics to the regional lymph nodes which contribute to the inflammatory response. From the lymph nodes, Salmonella spp. may travel via the efferent lymph vessels and drain into the circulatory system. They are filtered out of circulation via the reticuloendothelial system, usually by the spleen and liver. Release of endotoxin into the circulation may account for many of the systemic effects of disease including fever and vascular damage. Thrombosis of the small vessels may lead to ischemic necrosis of the extremities and tips of the ears, particularly with S. choleraesuis infection in swine. Failure to contain the infection will result in septicemia resulting in pneumonia, meningitis and septic arthritis (Wray and Sojka 1977).

Upper respiratory infection

It has suggested that infection of the upper respiratory tract may influence the outcome of infection. Aerosol experiments in chickens and mice have shown that infections with Salmonella spp. can be achieved more regularly via the lungs than by oral inoculation (Clemmer et al. 1960; Darlow et al. 1961).

Pneumonia associated with S. choleraesuis infection has been previously described (Baskerville and Dow 1973) and a recent increase in S. choleraesuis associated pneumonia has been reported (Turk et al. 1992). It is unclear whether this predilection for the lung is due solely to the pathogen, poor ventilation in large confinement buildings or some combination of these and other factors. Experimental infection models have not provided good answers because positive lung samples have been regarded as an artifact of intranasal or per os inoculation. However, Fedorka-Cray et al. (1995) has demonstrated that the lung is a primary site of colonization following intranasal inoculation of esophageotomized pigs. Additionally, Gray et al. (1995c) has also demonstrated that the lung is colonized in swine that are naturally exposed to pigs infected with S. choleraesuis. These data illustrate that lung colonization is not an artifact of experimental inoculation.

Swine have a large number of alveolar macrophages in the lung (Winkler and Cheville 1987). Fedorka-Cray et al. (1995) hypothesized that swine alveolar macrophages may have an impaired ability to contain Salmonella spp. within the early hours after infection. However, once uptake has occurred, the alveolar macrophages may act as a vehicle for dissemination of
*Salmonella* spp. (Fedorka-Cray et al. 1995).

**Virulence factors**

Many potential virulence factors have been identified for *Salmonella* spp. but few have been tested critically for their contribution to virulence. It has been estimated that *Salmonella* spp. possess over 200 virulence factors, only a fraction of which have been characterized (Curtiss 1994). Many studies have relied on *in vitro* data to draw their conclusions. This makes it difficult to develop meaningful extrapolations for human and animal disease. In addition many studies utilize mice as a model for disease and these results often cannot be repeated in other hosts.

Several serovars have been shown to produce enterotoxins specifically cholera-like toxin (Prasad et al. 1990, 1992). Very little is known about this toxin as it relates to the pathogenesis of *Salmonella* spp. If it acts similarly to cholera toxin, the B subunit of the protein will bind to the G_{M1} ganglioside on the membrane of intestinal epithelial cells after which the A subunit is internalized causing activation of cAMP and prostaglandin synthesis. These changes would result in fluid and electrolyte secretion (Falkow and Mekalanos 1990).

A common feature of *Salmonella* spp. induced enteritis is severe damage to intestinal epithelial cells likely the result of a cytotoxin. At least three cytotoxins have been identified. A wide variety of serovars possess a heat-labile cytotoxin described by Ashkenazi et al. (1988). Another cytotoxin is a low molecular weight, membrane associated toxin which has not been characterized (Reitmeyer et al. 1986). A third toxin, described by Libby et al. (1990), appears to be present in nearly all *Salmonella* spp., *Shigella* and enteroinvasive *E. coli*. This cloned protein is a 26 kDa cell-associated hemolysin and its role in virulence is under study.

The LPS of *Salmonella* spp. is a major determinant of host specificity and virulence. The intact LPS affords resistance to phagocytosis and killing by macrophages and complement-mediated killing (Saxen et al. 1987; Robbins et al. 1992). In addition it has been shown that LPS is a major contributor to survival of *Salmonella* spp. in the intestinal tract (Nnalu and Lindberg 1990). The LPS component of *Salmonella* spp. also contributes to vascular damage and thrombosis. Endotoxic properties result in fever, disseminated intravascular coagulation, circulatory collapse and endotoxic shock associated with salmonellosis (Takeuchi and Sprinz 1967; Clarke 1985).

Motility provided by flagella appears to be important for invasion for some, but not all, serotypes of *Salmonella*. Regardless of the other contributions the flagella may make, their presence increases the probability that the organism will come in contact with an epithelial cell. It has been shown that strains with polar rather than peritrichous flagella have increased ability to come in contact with, and potentially invade, epithelial cells (Jones et al. 1992).

A siderophore has been identified in *S. typhimurium* called enterobactin (Benjamin et al. 1985). This protein does not appear to be necessary for full virulence and the importance of the protein may be relative to the amount of extracellular growth which occurs. Interestingly, pigs infected with *S. choleraesuis* have a reduction in serum iron, total-iron binding capacity and transferrin. The intracellular environment is low in iron and it has been suggesting that *S. choleraesuis* has a nonsiderophore mechanism for scavenging iron (Clarke and Gyles 1993).

Finally, heat shock proteins have been shown to be produced by *S. typhimurium* inside murine macrophages. Mutants which are defective in this ability to produce these proteins are less virulent in mice and do not survive well in macrophages (Falkow and Mekalanos 1990).
DISEASE IN SWINE

Associated serotypes
Clinical swine salmonellosis can be separated into two syndromes. *Salmonella typhimurium* is associated with enterocolitis, while *S. choleraesuis* is usually associated with septicemia. In the United States clinical swine salmonellosis is almost solely due to infection with *S. typhimurium* or *S. choleraesuis*. Clinical disease has also been associated with *S. typhimurium*. This serotype is difficult to isolate and because of this difficulty may be responsible for more outbreaks than it is directly associated with by culture (Wilcock and Schwartz 1992; Glock 1994). In addition, there have been reports of both *S. dublin* (Lawson and Dow 1966) and *S. enteritidis* (Reynolds et al. 1967) causing disease in swine. In contrast, other countries see clinical disease from many serotypes and *S. choleraesuis* may or may not be one of them (Nielsen 1995).

The vast majority of *S. choleraesuis* outbreaks in swine are due to the H2S producing variant *kunzendorf* (Wilcock and Schwartz 1992). However, the non-H2S producing *S. choleraesuis* has been as high as number 2 in the top 10 most common *Salmonella* isolates from swine in a given year (Ferris and Thomas 1993).

Populations affected
Intensely reared weaned pigs are most often affected by *Salmonella* infection. In general, *S. typhimurium* tends to cause disease in young pigs from six to twelve weeks of age. Disease from this serotype is rare in adult animals; however, infection is not. *Salmonella choleraesuis* causes disease among a wider range of ages. Mortality tends to be higher in younger rather than older pigs, while morbidity is often regardles regardless of age. Disease from *S. choleraesuis* in the adult is not a common occurrence. However, if a susceptible population is exposed, the animals will be affected significantly (Wilcock and Schwartz 1992). It is not known how common subclinical infection is in the adult. Normally only moribund, suspect cases are cultured for *S. choleraesuis*. In suckling pigs disease is distinctly uncommon but infection is not (Gooch and Haddock 1969; Wilcock et al. 1976). The occurrence of salmonellosis in suckling pigs is rare, presumably because of lactogenic immunity, while neonatal swine are susceptible to oral challenge with salmonellae and develop disease similar to that observed in weaned pigs (Wilcock and Olander 1978).

Septicemia
The septic form of porcine salmonellosis is usually caused by *S. choleraesuis*. Affected pigs are inappetent, lethargic and febrile with temperatures up to 107°F. Respiratory signs may consist of a shallow moist cough and diaphragmatic breathing. Clinical signs first appear after 24-36 hours of infection (Reed et al. 1986). Often, producers will find the first evidence of disease as dead pigs with cyanotic extremities and abdomens. In most outbreaks, mortality is high and morbidity is variable but generally less than 10% (Reed et al. 1986; Wilcock and Schwartz 1992). Diarrhea is normally not a feature of *S. choleraesuis* infection until at least the fourth or fifth day of infection. It may last from five to seven days after onset if chronic reinfection is not occurring.

Gross lesions include colitis, infarction of gastric mucosa, swollen mesenteric lymph nodes, splenomegaly, hepatomegaly and lung congestion. Random white foci of necrosis are often observed on the liver (Reed et al. 1986; Wilcock and Schwartz 1992).

The microscopic lesion which is most often associated with *S. choleraesuis* in swine is the paratyphoid nodule. This lesion can be viewed in the liver as clusters of histiocytes amid foci of acute coagulative hepatocellular necrosis and corresponds to the white foci seen grossly (Lawson and Dow 1966). Other lesions may include fibrinoid thrombi in venules of gastric mucosa, cyanotic skin and glomerular capillaries. Swelling of histiocytes and epithelial cells typical of gram negative sepsis, as well as hyperplasia of reticular cells of the spleen and lymph nodes are often observed (Wilcock et al. 1976).
Enterocolitis

Salmonella spp. enterocolitis in pigs is typically associated with S. typhimurium infection and occasionally with S. choleraesuis infection. In contrast to the septicemic disease, the initial sign of infection is often a watery yellow diarrhea. Infected pigs are inappetent, febrile and lethargic. Mortality is usually very low, however, morbidity can be very high within a few days after infection (Wilcock and Schwartz 1992).

The major gross lesion at necropsy is focal or diffuse necrotic colitis and typhilitis. Mesenteric lymph nodes are greatly enlarged. Intestinal lesions develop as red, rough mucosal surfaces that may also have gray-yellow debris. Colon and cecal contents are bile stained and scant, often with black or sand-like gritty material on the surface. Intestinal necrosis may be seen as sharply delineated button ulcers often associated with resolving lesions (Wood and Rose 1992, Wilcock and Olander 1978; Wilcock and Schwartz 1992). In cases of S. typhimurium enterocolitis, the liver and spleen are not enlarged except by terminal congestion (Wilcock and Schwartz 1992).

Histopathologic examination reveals necrosis of cryptic and surface enterocytes which may be local or diffuse. The lamina propria and submucosa contain macrophages and lymphocytes with neutrophils observed only in the very early stages of disease. It is not uncommon to see lymphoid atrophy or regenerative hyperplasia associated with this disease (Wilcock et al. 1976, Jubb et al. 1985, Reed 1986).

Epidemiology

General introduction

Members of the genus Salmonella are extremely ubiquitous in nature, recovered from nearly all vertebrates as well as insects and are often referred to as universal pathogens (Taylor and McCoy 1969; Falkow and Mekalanos 1990). Taken as a whole, it is useful to group Salmonella spp. into three groups on the basis of host-adapted preference. The first group are Salmonella serotypes highly adapted to humans. The prototype of this group is the typhoid bacillus, S. typhi. The second group are Salmonella serotypes highly adapted to specific hosts other than humans. Some examples of this group are S. pullorum or S. gallinarum which are adapted to avian hosts or S. typhimurium and S. choleraesuis which are adapted to swine. In addition, S. abortusovis is a serotype highly adapted to sheep and is a major cause of abortion in ewes. There are also serotypes such as S. dublin which is viewed primarily as a pathogen of cattle but is often found in other hosts (LeMinor 1984). However, some serotypes in this second group can cause severe disease in humans which may result in high mortality. This has been observed following infection by S. choleraesuis (Cherubin 1980). The third group of salmonellae would be those with a broad host range. Most Salmonella spp. belong to this category and S. typhimurium is the best known serotype of this group. It is the serotype most frequently associated with gastroenteritis worldwide (Falkow and Mekalanos 1990).

Salmonella in pork products

Wilcock and Schwartz (1992) consider the epidemiology of Salmonella in swine as two relatively separate problems: 1) The contamination of pork carcasses and retail products with Salmonella spp. and 2) salmonellosis as a disease of pigs. They also point out that failure of prevalence surveys to distinguish the two conditions has led to considerable confusion about the etiology and epidemiology of clinical salmonellosis in swine. It should be noted that infection of swine and swine products by a wide variety of serotypes is common, but clinical disease caused by serotypes other than S. typhimurium or S. choleraesuis is distinctly uncommon.

Due to the potential threat of foodborne illness in humans resulting from consumption of contaminated pork, it is appropriate that we briefly consider this subject. The results of these
studies will not be discussed in depth here. It is accepted that the infected pig leaving the farm is most often considered the original source of abattoir infections. Also of importance is that S. choleraesuis is rarely associated with contamination of carcasses and pork products. As mentioned earlier, S. choleraesuis is a host adapted serotype that rarely infects humans; however, in cases where it has infected humans, it presents a disease of grave consequence that is difficult to diagnose and treat (Cherubin 1980).

In contrast, the top 10 serotypes isolated from swine in 1994 (Ferris and Thomas 1994) include at least 3 (S.typhimurium, S. heidelberg, S. agona) of the top 10 serotypes commonly associated with human disease (Bean and Griffin 1992). This indicates that serotypes which commonly cause disease in humans may be closely associated with pork and pork products.

**Distribution and prevalence**

Salmonellosis as a disease in swine occurs worldwide but varies markedly in estimated prevalence and mortality as there seems to be variation in prevalence between serotypes. Some of this variation can likely be explained by the virulence of the specific strains endemic to an area or from the genetic variation of breeding stock. Investigators have added to the confusion in this area. Often reviews report epidemiologic data from slaughterhouse or federal surveillance studies which are unsupported by clinical or pathologic criteria for salmonellosis (Wilcock and Schwartz 1990). There is also marked variation in the prevalence of Salmonella spp. responsible for disease production in data reported from diagnostic laboratories. There could be many explanations for this amount of variation. Of particular concern are variations in bacteriologic culture methods utilized to isolate the organism. Salmonella typhimurium is much less difficult to isolate than S. choleraesuis because it grows readily in all of the standard selective media used, whereas host adapted strains often require more specialized media (Ewing 1986).

Overall, regardless of animal species, the number one Salmonella isolate is S. enteritidis. Ferris and Freerichs (1990) found that S. choleraesuis has been, and is currently, the second most frequently isolated Salmonella spp. from all animal sources in the United States since 1979. The isolation rate was greater than 99% from swine when compared to all other animals.

In the late eighties and early nineties, the reported isolations of salmonellosis due to S. choleraesuis were increasing. One laboratory reported 256 isolations in 1981 with gradual increases to 788 in 1989. In 1989 S. choleraesuis was isolated from >95% of swine salmonellosis cases while S. typhimurium represented 4% of cases (Schwartz 1990). Causes for the increase are unknown. Recent reports indicate the trend may be declining (Ferris and Thomas 1994).

**Cost**

Owen (1990) estimated that the cost of salmonellosis as a disease to Iowa swine producers ranks second to swine dysentery caused by Serpulina hyodysenteriae. The National Animal Health Monitoring Survey estimated that swine salmonellosis is responsible for 28 million dollars in annual production losses in Iowa and 100 million in losses nationwide (Schwartz 1990). There are no estimates of costs associated with subclinical infections of Salmonella in swine.

**Source of infection**

As previously noted, Salmonella is ubiquitous in nature. However, if one recognizes that S. choleraesuis is the most frequent porcine isolate, but is rarely isolated from swine feeds or non-porcine salmonella reservoirs, the conclusion must be drawn that the infected shedding pig is the source of new infections (Wilcock and Schwartz 1992). It has been shown that experimentally challenged pigs can shed up to 10^6/g of S. choleraesuis (Smith and Jones 1967) and 10^7/g of S. typhimurium (Gutzmann et al. 1976) in the feces. The challenge inocula used in these studies were as high as 10^11 cfu which seems to be an inoculum unlikely found in the environment. The minimum infective dose for either S. choleraesuis or S. typhimurium has not been established. Often investigators infect swine with doses of 10^8-10^11 cfu. Fedorka-Cray et al. (1994)
demonstrated that pigs infected with $10^4$ CFU of *S. typhimurium* will develop a short term carrier state. Gray et al. (1995b) demonstrated that experimental infection of pigs with $10^3$ CFU of *S. choleraesuis* will be cleared with no apparent shedding or clinical signs. In contrast, a dose of $10^6$ CFU results in persistent infection for at least 9 weeks.

Natural transmission studies with *S. typhimurium* in swine have indicated that subclinical carriers develop when naive swine are exposed to a population of swine shedding $<10^3$ CFU (Fedorka-Cray et al. 1994). Gray et al. (1995c) demonstrated that natural exposure of *Salmonella*-free swine to a population shedding $10^3$ CFU *S. choleraesuis*/g of feces will result in a severe clinical outbreak with some of the population carrying the organism for at least 12 weeks. In comparison to experimental models, the naturally exposed swine would have needed to ingest between 250 g ($10^6$ CFU dose) and 25000 g ($10^8$ CFU dose) of feces each to manifest the severe clinical signs observed in this experiment. This amount of coprophagia is unlikely. These data suggest that during natural transmission, the infectious dose of *S. choleraesuis* is much lower than experimental models have previously described. Swine may also be exposed to a large dose of *S. choleraesuis* by a mechanism other than fecal-oral transmission.

**CARRIER STATE AND SHEDDING**

*Species other than swine*

Although *Salmonella* spp. may survive for long periods in the environment, it is widely believed that the carrier animal is the major source of infections for both animals and humans (Wray and Sojka 1977).

The carrier state is defined as the absence of evidence of disease in animals that are able to transmit infection to susceptible individuals (Thrusfield 1986). Carrier animals develop as a result of the interaction of several factors including the serotype of *Salmonella*, age of the animal, and number of bacteria ingested. Young cattle often shed *Salmonella* only during convalescence, whereas adults are more likely to become chronic shedders. In addition a low dose which is insufficient to cause disease may result in a carrier state (Wray and Sojka 1977).

In cattle various types of carrier states have been identified (Wray and Sojka 1977). The active carrier state may follow recovery from clinical disease and cattle may excrete *Salmonella* spp. for months or years in the milk and/or the feces. The active carrier state often persists in the presence of high serum antibody titers to *Salmonella* O and H antigens.

Passive carriers are described as cattle which ingest *Salmonella* spp. and pass the organisms through the intestine into the feces with little or no invasion of the mesenteric lymph nodes. These animals cease shedding *Salmonella* spp. shortly after they have been removed from the contaminated environment (Wray and Sojka 1977). Latent carriers are cattle which have deep tissue infection with *Salmonella* spp. but do not excrete the organism in their feces (Wray and Sojka 1977). Excretion may be reactivated by unknown mechanisms.

Treatment of adult animals with antibiotics during the course of disease is ineffective in eliminating the carrier state in cattle infected with *S. dublin* (Wray and Sojka 1977). It is widely accepted that the antibiotic treatment of humans following infection with *S. typhi* (typhoid) is contraindicated as it prolongs the carrier state (Askerkoff and Bennett 1969).

Certain stress factors have been shown to promote activation or reactivation of clinical signs and shedding in *Salmonella* carrier cattle. These factors include, but are not limited to, transportation of animals, overcrowding, corticosteroids, parturition and concurrent infection. Tannock and Smith (1971b) described the carriage of *S. typhimurium* in sheep for up to 6 weeks after intranasal inoculation. When the same inoculum was given orally by use of a gelatin
capsule a prolonged carrier state was not observed. Tannock and Smith (1971a) also compared the effect of route of inoculation on the carrier state in mice. They concluded the upper respiratory tract provides a focus of infection. When the inoculation route is intranasal, a carrier state results for at least 6 weeks. As observed in sheep, the gastric route of inoculation did not result in development of a carrier state.

**Carrier state in swine**

After experimental challenge of swine with *S. typhimurium*, *Salmonella* could be isolated from the feces daily for the first 10 days and frequently over 4-5 months. Four to seven months post challenge carrier animals were necropsied and greater than 90% of the pigs were positive for *S. typhimurium* in the mesenteric lymph node, tonsil, cecum or feces (Wilcock and Olander 1978; Wood et al. 1989). In an unrelated experiment it has been shown that a sub-clinical, undetectable infection can progress to a high level of shedding after the occurrence of stressful events such as farrowing or transport to slaughter (Wilcock and Schwartz 1992).

In contrast to this minimal understanding of the carrier state of *S. typhimurium* in swine, the duration of shedding and the locations of organisms for *S. choleraesuis* in carrier swine has not been studied until recently. Gray et al. (1995a) has recently described the carrier state of *S. choleraesuis* in swine. These data demonstrate that a subclinical carrier state exists for at least 12 weeks after experimental challenge. The tissues in which *S. choleraesuis* can most commonly be found in carrier swine are the ileocolic junction, ileocolic lymph node, cecal contents, tonsil, lung and colon, regardless of route of inoculation. Swine can also shed *S. choleraesuis* in the feces sporadically throughout the 12 week period (Gray et al. 1995a). A dose dependant effect on persistence of *S. choleraesuis* in swine has also been observed (Gray et al. 1995b). Lower challenge doses such as 10^3 CFU may be cleared by pigs. In contrast, a moderate dose (10^6 CFU) may result in persistent infection for at least 9 weeks. High challenge doses (10^9 CFU) have been shown to result in long term carriers which may be related to an observed lymphocyte immunosuppression.

Recent experiments have indicated that *S. choleraesuis* which have been shed from infected swine can survive for at least 3 months in a wet fecal slurry and at least 6 months in dry, desiccated feces (Gray et al. 1995d). This indicates the importance of decontamination of the environment when reduction of *Salmonella* spp. is a goal.

The influence of antibiotics on the frequency and duration of shedding of *Salmonella* in swine is not well understood. However, it is known that antibiotics do not affect the magnitude or intensity of shedding of *S. typhimurium* in swine (DeGeeter et al. 1976; Jacks et al. 1988). Conversely, it has been shown that antibiotics may reduce the magnitude and duration of shedding of *S. choleraesuis* (Jacks et al. 1981).

**IMMUNITY AND VACCINATION**

**Introduction**

There is continual debate over the importance of the humoral versus the cell-mediated immune response following *Salmonella* infections. This controversy stems from the pathogen's ability to reside successfully in both an intracellular and extracellular environment. Taken as a whole, these data suggest that both humoral and the cell-mediated response are important in resistance to *Salmonella* spp. infection. At the present time, there is a lack of information regarding the immune response in swine following *Salmonella* spp. infection.

**Humoral immunity**

Antibodies against *Salmonella* are common in sera following exposure to the pathogen. Passive transfer of antibodies against *Salmonella* spp. to offspring is observed (Royal 1968).
Antibody can provide protection through opsonization of the pathogen, neutralization of toxins and initiation antibody dependent cell-mediated cytotoxicity. Many antigens from *Salmonella* have been shown to induce antibodies including LPS (Jimenez-Lucho and Leive 1990), proteins (Foulaki et al. 1989; Saxen et al. 1986; Udhayakumar and Muthukaruppan 1987) and ribosomal fractions (Eisenstein 1975).

The importance of humoral immunity in protection from *Salmonella* infection is discussed by Eisenstein and Sultzter (1983). The experiments conducted in mice show increased host survival following challenge, increased duration of survival and passive protection against homologous strain challenge. Evidence suggests that specific antibodies in the colostrum of cows vaccinated with *Salmonella* may interact with organisms in the lumen of the gut of calves and influence the outcome of infection (Royal 1968).

Natural and antibody-dependent antibacterial mechanisms may be important in defense against *Salmonella* spp., particularly in the gastrointestinal tract. Secretory IgA is also found in the intestine of animals that have recovered from disease or that have been vaccinated orally (Clarke and Gyles 1994; Stabel 1993). Intestinal antibodies constitute the first line of specific immune defense to organisms which enter the gastrointestinal system.

The amount of specific antibody in serum has not been shown to correlate with protection against experimental challenge of calves vaccinated with either a live attenuated mutant or heat-killed preparations of *Salmonella* spp. (Habasha 1981; Lindberg and Robertsson 1983). In addition, not all animals which are infected or immunized with *Salmonella* develop titers to the organism even though they show increased survival from subsequent challenge (Clarke and Gyles 1994). Recently, antibody titers to nontyphoidal *Salmonella* O-antigen have been suggested to confer protection against challenge (Robbins et al. 1992).

**Cell-mediated immunity**

*Salmonella* is a facultative intracellular pathogen which can evade antibody in the intracellular environment. This suggests that a strong cellular response is needed for pathogen elimination (Sell 1987). Infection with *Salmonella* has been shown to effectively induce cell-mediated immunity (Hanna et al. 1979a; Hanna et al. 1979b; Hassan and Curtiss 1990; Jones et al. 1991). Transfer of sensitized T cells confers protection, whereas transfer of macrophages and B cells are not protective in the absence of T cells. Delayed-type hypersensitivity develops in animals with natural and experimental salmonellosis (Robertsson et al. 1982a,b; Lindberg and Robertsson 1983).

Generally, the cell-mediated immune response correlates well with protection in calves and mice immunized with live attenuated vaccines and subsequently challenged with large numbers of *Salmonella* spp. (Habasha 1981; Lindberg and Robertsson 1983; Eisenstein and Sultzter 1983). Cross-protection has been demonstrated for *S. typhimurium* and *S. dublin* and may be attributed to shared O-antigenic components and porin antigens (Habasha 1981; Lindberg and Robertsson 1983).

Interestingly, investigators have demonstrated that live vaccines were superior to killed bacteria in providing increased host survival which was facilitated by elimination of the organism from the spleen and liver (Collins 1974; Eisenstein and Sultzter 1983). Using a mouse model of typhoid fever the immunity induced by an araA mutant of *S. typhimurium* was attributed to natural killer cell activity that was observed in the early stages of disease. It was suggested that these cells may also contribute to host defense in the later stages of disease (Schafer and Eisenstein 1992).

**Mucosal immunity**

Mucosal surfaces are the major sites in the body in which antigens are encountered. Throughout life, they are continuously bombarded by antigens, whether they are ingested food
particles, microbes such as Salmonella, toxins, parasites, or allergens. Most infectious diseases develop on mucosal surfaces and in many the organism is limited to these sites. To combat this constant threat, vertebrates have developed a complex mucosal immune system that undertakes the task of limiting infections without interfering with the function of the fragile mucosal tissue (Klein 1989).

The major humoral immune factor at these sites is locally produced secretory IgA antibody (Mestecky 1987). It has been estimated that 65 to 90% of immunoglobulin-producing cells produce IgA and 75% of the total immunoglobulin produced in humans is IgA (Michalek et al. 1995).

An antigen that has come in contact with the mucosal surface must cross the epithelium to reach the lymphoid tissue in the lamina propria. On some mucosal surfaces, this crossing is affected by specialized cells overlying the lymphoid follicles (Michalek et al. 1995). In the intestine, and probably also in the respiratory system, crossing the epithelium is regulated by M cells. On the side opposite the luminal surface, lymphocytes attach themselves closely to the M cells. The M cells bind antigen on the luminal side, endocytose it and then exocytose the antigen to the lymphocytes on the opposite side. The M cells differentially bind antigens excluding commensal organisms and food antigens. Some invasive pathogens such as Salmonella spp. can use this transport mechanism to their advantage which results in access to macrophages and lymphocytes in which they can survive (Klein 1989).

The M cells transport material in both directions. Lymphocytes, for example, have been seen moving through M cells by diapedesis and then entering the lumen of the gut. It has been hypothesized that most of the lymphocytes found on the luminal surface of the gut mucosa may have reached their destination by this manner (Klein 1989). This may be the mechanism by which swine challenged with S. typhimurium by mechanisms completely excluding oral gastric exposure are observed to have S. typhimurium positive intestinal tissues after only a few hours (Fedorka-Cray et al. 1995). It should be noted that the M cells take up antigens, but are unable to carry out the crucial presentation step which is required to initiate an immune response because they lack class II Mhc molecules and are unable to process the antigen for presentation (Klein 1989). Therefore, the M cells must release the antigen to the mucosal associated lymphoid tissue (MALT) where presentation can take place.

The collaboration of B lymphocytes with T helper lymphocytes (Th) may occur in the MALT or the draining lymph nodes. The B cells that encounter antigen and receive the necessary stimulation from Th cells leave the MALT without secreting antibody. As they mature and begin secreting antibody, they home back to the various MALTs where they settle back into the lamina propria (Bergmann 1986, Ogra and Karzon 1969). If the B cells encounter the same antigen again they begin to produce antibody resulting in a mucosal antibody response. What determines the B cells specificity to the MALT sites remains unclear. However, the homing of the B cells for the various MALTs is one characteristic of the mucosal immune system; another characteristic is the dominance of IgA-producing B-cells over any other B lymphocytes although IgM, IgG and IgE antibodies are also produced but in lesser amounts (Klein 1989).

The cells found in the MALT include lymphocytes, natural killer cells, macrophages, mast cells and eosinophils. At least some of the lymphocytes, the intraepithelial lymphocytes, seem to be unique in the mucosae. Among this population about 15% are T lymphocytes. There is a dispute regarding the remaining 85% of intraepithelial lymphocytes. The lamina propria contains true lymphocytes, both T and B cells, providing the necessary components for the initiation of an immune response in this area (Klein 1989).

Mucosal antibody responses to Salmonella antigens have been shown in swine after challenge (Gray et al. 1995 a,c) and vaccination (Stabel et al. 1993). However, there have not
been any studies providing a correlation of mucosal antibody response and increased resistance to salmonellosis in swine despite the critical importance of mucosal immunity.

**Vaccination**

It is generally accepted that live attenuated, orally-administered *Salmonella* vaccines provide the best protection against *Salmonella* infection. The superior protection achieved in comparison to killed *Salmonella* bacterins and subunit vaccines is generally attributed to the ability of live vaccines to stimulate a more effective cell-mediated immune response. Oral administration allows the attenuated mutant to utilize natural routes of infection which facilitates the crucial step of antigen presentation to lymphocytes in the gut-associate lymphoid tissue. These events induce the production of secretory IgA on mucosal surfaces (Clarke and Gyles 1994).

Recently the development of specific nonreverting mutations to construct both homologous and heterologous vaccine vehicles with multiple attenuating mutations has been achieved (Chatfield et al. 1992).

A mutation in the *galE* region in *S. typhi* results in a deficiency in UDP-glucose-4-epimerase, the enzyme which converts UDP-glucose to UDP-galactose, an essential component of *Salmonella* spp. smooth LPS (Levine et al. 1989). In several large trials utilizing human subjects this mutant has appeared to be very efficacious. Because of this success, this mutation has been employed for many *Salmonella* serotypes including *S. typhimurium* (Nnalue and Lindberg 1990). However, the *galE* mutation was not successful when utilized in *S. choleraesuis*. The O antigen of *Salmonella* serogroups are the main component of host specificity and facilitate survival in the gastrointestinal tract and entry onto deeper tissues (Nnalue and Lindberg 1990). The *galE* mutation in *S. choleraesuis* does not reduce virulence in swine. This is due to the fact that galactose is missing from the oligosaccharide repeating unit of the O antigen side chain of *S. choleraesuis* (Nnalue and Stocker 1986).

Another common attenuation involves the creation of auxotrophic mutants that require metabolites not available in animal tissues. Aromatic mutants, which have a complete block in the aromatic biosynthetic pathway have a requirement for aromatic metabolites such as para-aminobenzoate and 2,3-dihydroxybenzoate. Oral vaccination with *aroA*, *aroD* mutants in mice and calves has been effective in reducing disease and have been shown to be safe (Hook 1990; Robertson et al. 1983; Smith et al. 1984).

Mutations in global regulatory pathways have also been a popular means of attenuation. Several studies have utilized strains with deletions (∆) in the genes for adenylate cyclase (*cya*) and for cAMP-receptor protein (*crp*). Cyclic AMP and cAMP-receptor protein regulate at least 200 genes, many of which are required for breakdown of catabolites. *Salmonella* with deletion mutations in the *∆cya ∆crp* genes have been shown to be safe and effective in eliciting protective immunity in mice, chickens and pigs (Coe and Wood 1992; Curtiss and Kelly 1987; Stabel et al. 1990; Stabel et al. 1991). A large study evaluating the safety and efficacy of a battery of *S. choleraesuis cya, crp* isogenic mutants in mice indicates that several of these strains are protective and safe (Kelly et al. 1992).

Recently a *S. choleraesuis* strain which has been cured of the 50 kb virulence plasmid has been shown to safe and efficacious in swine (Kramer et al. 1991). The nonspecific mutation was obtained by repeated passage through porcine neutrophils. The plasmidless variant lacks the ability to invade Vero cell monolayers and porcine neutrophils as well as having increased resistance to killing by H$_2$O$_2$ and phagocytic killing by porcine neutrophils (Roof et al. 1992).
DETECTION OF SALMONELLA

Culture

A great interest has developed in the animal production and food processing industries to create and evaluate new methods to detect, either directly or indirectly, the presence of Salmonella spp.. Traditional culture methods are slow, cumbersome, expensive and require considerable manpower to complete. However, the culture of Salmonella is the standard by which all other methods are measured. Recovery of the organism is the only means by which definitive serotyping can be achieved. In addition, the isolation of the organism serves as an invaluable source of epidemiologic data which cannot be overlooked.

It is always advisable to employ enrichment culture in the examination of various kinds of specimens for Salmonella spp. The first step in the culture process should include an assessment of the competing flora and the physical state of the Salmonella in the sample. For example, for a pellet feed sample which has been heated and dried in the processing step there will likely be relatively few competing flora and the Salmonella may be in a desiccated state. This warrants use of a non-selective nutrient broth (Ewing 1986). In contrast, fecal samples contain large numbers of competing flora and the Salmonella may be in any stage of growth indicating the use of selective media for enrichment (Ewing 1986).

The media suggested for enrichment of Salmonella spp. in fecal specimens from carriers or suspected carriers are tetraithionate broth and selenite F broth (Ewing 1986). Many modifications have been made to these media and the application should be considered when choosing a medium for a specific purpose. Another useful medium for the enrichment of Salmonella spp. is Rappaport medium (Vassiliadis 1983), which utilizes malachite green and magnesium chloride as selective agents. However, this medium is easily overloaded when used as an initial enrichment and care must be exercised when developing an inoculation plan (1986).

Smith (1952) found it absolutely necessary to utilize media other than tetraithionate broth and selenite F broth for the isolation of S. choleræsis, both of which have been reported to be toxic for S. choleræsis. The same caution is warranted when any host adapted serotype is suspected as many of the of the host adapted serotypes do not grow well in the highly selective Salmonella media, including S. typhi (LeMinor 1984). It has been suggested that this may explain the infrequent isolation of S. choleræsis in swine associated epidemiologic surveys (Ewing 1986). When possible, a combination of enrichment media should be employed and may include GN-Hajna broth and tetraithionate broth for the isolation of host adapted serotypes as well as broad host range Salmonella spp. (Ewing 1986).

Many plating media have been devised for the isolation and differentiation of Salmonella spp. Plating media for use of isolation and differentiation of Salmonella spp. and other members of the genus Enterobacteriaceae may be divided into categories according to their selectivity.

Differential media, with little selectivity for enterobacteriaceae, are used with some frequency. This group includes MacConkey agar on which the lactose negative Salmonella spp. appear as white colonies. All Salmonella spp. grow well on MacConkey agar (Ewing 1986). Moderately selective differential media includes Shigella-Salmonella agar and Hektoen Enteric (HE) agar. Hektoen Enteric agar is often considered a standard by which other Salmonella isolation media are measured (Dusch and Altweeg 1995). Again, all serotypes of Salmonella grow well on moderately selective differential media (Ewing 1986).

The final category is the highly selective media which include brilliant green (BG) agar. The BG agars are very popular and can be considered as a one purpose medium for the isolation of Salmonella spp. Salmonella appear as smooth pink colonies on BG agar. Only a few other genus of bacteria can also appear as pink colonies similar to Salmonella and include pseudomonads,
aeromonads, proteus and late lactose fermenting E. coli. Although these agars are very useful in the isolation of Salmonella spp. some serotypes such as S. typhi do not grow well on these agars (Ewing 1986).

A recent study compared HE agar, Rambach agar, SM-ID medium, xylose-lysine-Tergitol 4 agar (XLT4), novobiocin-brilliant green-glycerol-lactose agar (NBGL) and modified semisolid Rappaport Vassiliadis medium (MSRV) for the isolation of Salmonella spp. The test of these relatively new media found MSRV to be the most sensitive and specific but it was also the most difficult to use. The XLT4 plates were found to be as sensitive as HE with improved specificity. The other media did not perform as well (Dusch and Altwegg 1995).

In all cases, pooled fecal samples are preferred over rectal swabs for the detection of Salmonella-carrier pigs (McCall et al. 1966).

**Enzyme linked immunosorbent assays**

Enzyme linked Immunosorbent Assays (ELISA) can be used to detect either the organism or a humoral immune response to the organism. Assays utilized to detect microorganisms in food and feed stuffs are gaining widespread use in the industry and are called antigen-capture ELISA. Whereas culture may take 3-7 days to identify the organism, ELISA can detect the organism in a much shorter period of time, usually 1 day or less. However, the reliability of some of these assays is questionable. In general, the cleaner the sample the better the assay will perform. Usually feces does not test as well as food and feedstuffs. Feng (1992) listed and described several commercial rapid screening assays. Several antigen capture immunoassays have been utilized to detect Salmonella spp. in swine feces (Araj and Chugh 1987; Lambiri et al. 1990; van Poucke 1990). They have the same disadvantage of many ELISA tests in that they require 10^4-10^5 CFU of Salmonella per ml to detect the organism (Dzieczak 1987). In order to achieve these numbers, a time consuming and expensive concentration protocol or a lengthy pre-enrichment must be employed. Some investigators have had success utilizing rapid enrichment protocols to detect Salmonella spp. in swine feces (Cherrington and Huis in't Veld 1993a,b).

The second use of ELISA is to detect animals which have been, or are currently, infected with Salmonella spp. This procedure is not new, and was first described by Carlsson et al. (1972) to detect antibodies specific for Salmonella LPS. Several studies have focused on this approach and have found that LPS antibody titer can be an important diagnostic tool for detection of Salmonella infected cattle (Smith et al. 1989; Spier et al. 1990, 1991).

The detection of antibodies to the O antigen of Salmonella has also been utilized successfully in swine (Nielsen et al. 1994). The mixed ELISA utilizes LPS produced by the method of Westphal (1965) from either S. typhimurium or S. choleraesuis. The majority of swine produce high titers to the O-antigen which are present whether or not shedding can be detected (Nielsen et al. 1994). The test can be utilized as a herd test but is not suited as an individual pig test. Unfortunately, experimentally and naturally infected swine have been shown to have a titer to LPS for at least 12 weeks after exposure to S. choleraesuis even after clearing the bacteria (Gray et al. 1995a,c). This may result in a number of ELISA positive pigs which are no longer infected. It is unclear what effect vaccination has on the outcome of this assay. However, data indicates swine vaccinated with a commercially available modified live, plasmidless S. choleraesuis vaccine do not initiate a humoral immune response to S. choleraesuis antigens (Gray et al. 1995e). This would suggest that swine vaccinated with this strain would appear as noninfected pigs on a diagnostic test.

Another ELISA has been utilized to detect antibodies in Salmonella carrier swine employing a heat-extracted antigen (Kramer et al. 1994). The results from this study indicate that most pigs infected with S. typhimurium or S. choleraesuis have an antibody response to this antigen. This assay shows a correlation between higher magnitude of infectivity and a higher antibody response.
Polymerase chain reaction

The extraordinary ability of the polymerase chain reaction (PCR) to exponentially replicate a target DNA sequence has made it a very powerful tool in the armamentarium of the diagnostian, epidemiologist and molecular biologist. This assay is based on the ability of target (organism) specific primers which, through complimentary DNA base-pairing, anneal only to the target sequence. Thermostable DNA polymerase recognizes the template primer complex as a substrate which results in the simultaneous copying of both strands of the segment of DNA between the two annealed primers. The denaturation annealing and elongation steps take place in a cyclical fashion relying on the thermostability of the Taq-polymerase until the target sequence is amplified to detectable amounts (Ehrlich and Sirko 1994).

The PCR assay has been used to identify Salmonella spp. in food and clinical samples (Araj and Das Chugh 1987; Rahn et al. 1992; Cohen N.D. et al. 1993). However, obstacles in the detection of organisms include the presence of substances inhibitory to PCR (Rossen et al. 1992; Wilde et al. 1990) and the inability to detect <10³ CFU per gram of sample without preenrichment (Ehrlich and Sirko 1994). Investigators have improved detection methods in PCR assays by combining it with immunomagnetic separation (Widjojoatmodjo et al 1991; Widjojoatmodjo et al. 1992) or by enrichment culture (Stone et al. 1994).

FUTURE DIRECTION

Control of infection caused by serotypes other than S. choleraesuis is reliant on detecting the carrier pig, contaminated feed, or environmental sources of infection. Pigs are most likely to develop disease during periods of stress or when exposed to large numbers of salmonellae. The commingling and transport of weanling pigs from different sources to finishing farms enhances activation of latent carriers and assures exposure of stressed pigs to salmonellae (Allred 1972).

Because S. choleraesuis is rarely, if ever, isolated from feed or feed ingredients the source of new infections would seem to be limited to carrier pigs and facilities previously contaminated with this serotype. It is not uncommon for outbreaks to occur in facilities with good sanitation suggesting that stress is a likely contributor to disease.

Management practices which allow filling of grower and finisher rooms with single source and age pigs is beneficial. In addition, careful attention to good management practices such as proper animal density, dry comfortable pens, temperatures and adequate ventilation is critical (Wilcock and Schwartz 1992).

The detection of carrier animals through culture is sporadic at best because of the unpredictable nature of fecal shedding. Even repeated negative cultures may not ensure that a herd or individual is not a potential source of infection. Use of Salmonella serology will determine if the animal has had previous exposure to salmonella but this has not been shown to have relevance to the carrier status or to the predictable probability of shedding of an individual animal (Nielsen et al. 1994; Kramer et al. 1994). However, serological testing of herds will provide valuable information regarding the ongoing prevalence of infection in the herd and allow a measurement regarding success of control strategies. Additionally, refusal to introduce animals which have a positive titer will eliminate the introduction of potential carriers, but may also eliminate a proportion of the population which is not infected (Wilcock and Schwartz 1992).

In the United States monitoring herds for Salmonella spp. is not commonly practiced. However, other countries have had success with a monitoring and reduction program (Nielsen 1995). Current interest suggests that monitoring herds for the presence of Salmonella spp. in the United States may become more common. In addition, there is a great deal of research being conducted regarding the analysis of hazards and the critical control points for reduction of
Salmonella spp. in both the pre and post-harvest setting of swine production.

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SALMONELLA IN SWINE FEED AND FEED INGREDIENTS: A REVIEW

Isabel Turney Harris, DVM, PhD

Early Perspectives on Salmonella in Animal Feeds and Feed Ingredients

The presence of the salmonellae as contaminants of animal feeds and feed ingredients has been recognized for over 40 years. In his extensive review of Salmonella in poultry feeds, Williams (1981) traced the first reports of finding Salmonella in feed in the U.S. and Great Britain back to 1948. Attention was first focused on animal by-products used in feeds such as meat and bone meal, fishmeal, and meat scraps, when it was found that feed made from such products contaminated by Salmonella had the potential of introducing and spreading salmonellosis to domestic animals (Muller, 1952). As early as 1954, Denmark required that all imported meat and bone meal was to be resterilized before sale due to an association between the occurrence of Salmonella in poultry and the importation of large quantities of meat, bone, blood, and fish meal and bones (Muller, 1957). Vegetable products and finished feeds, both meal and pellets were soon found to harbor the bacteria as well (Grumbles and Flowers, 1961).

Studies reported on the detection of Salmonella in transport, handling and processing areas of animal feed production and other environmental sites (Pomeroy, 1958). Salmonella-free rendered feed ingredients were found to become recontaminated through poor handling and storage practices (Boyer et al., 1958), and Salmonella was transmitted to chicks in feed contaminated by the feces of rodents (Wilson, 1948). Heat treatment was recommended to eliminate Salmonella from meat meal (Kovacs, 1959).

Edwards (1958) was credited with first recognizing that efforts to eliminate salmonellosis from domestic animals must take into consideration the continual seeding of the animals through contaminated feedstuffs. A direct relationship was made between infection in turkey poult and Salmonella organisms in commercial feed fed to the poult, when the same serotype was found in unopened bags of the feed (Boyer et al., 1958).

Studies Concerning Feed as a Source of Salmonella in Pigs

Newell et al. (1959) in Northern Ireland, cited three studies to suggest that "the various Salmonella organisms so frequently isolated from fish and bone meal used for pig feedingstuffs might be related to Salmonella infections in pigs." A study was designed to examine by culture: pigs at slaughter, pigs on the farms from where the slaughter pigs originated, and the feedingstuffs on those farms in order to discover if there was a "chain of infection from a Salmonella contaminated product used in pig feeding to a food eaten by humans." On one farm the Salmonella serotypes found in fish meal and in pig fattening meal (S. infantis and S. schwarzenegger) were also found in pigs on the farm and pigs at slaughter. The authors commented on the difficulty of tracing Salmonella from a human case of salmonellosis back to the animals that consume feed and ultimately to the introduction of a batch of contaminated feedstuffs because of the time lag involved. Tracing the path of organisms forward from contaminated feedingstuffs to human food made from animal products was a more feasible course of action. In this study, Salmonella were isolated in much higher numbers from cecal contents than from cecal swabs at slaughter. The authors felt that this did not indicate that Salmonella infections found in slaughter pigs were due to cross contamination in the holding pens, because the percentage of positive rectal swabs taken from pigs at the farms (9%) was greater than that from cecal swabs (2%), but lower than cecal contents (23%) obtained at slaughter. However, Galton et al. (1954), believed a great deal of spread occurred in sale barns, during transportation, and in holding pens prior to slaughter. Their
group found a higher proportion of cecal swabs to be positive than post mortem rectal swabs and thought contamination might have occurred from the dehairing process.

Smith (1960) reported on the effect of giving feed naturally contaminated with Salmonella to eight week old pigs from a herd deemed free of Salmonella. The diet, containing contaminated fishmeal (50 organisms/100 g.) and bonemeal (700 organisms/100g.) was fed for 50 days during which time postmortem samples from sacrificed pigs and rectal swabs from the remaining pigs were cultured for the presence of Salmonella. The organism was demonstrated in the rectum of one pig after four days on the feed but only in the mesenteric lymph nodes after that. Salmonella was not found from rectal swabs until day 14 of the experiment for a total of 19 of 134 specimens collected during the 50 days. None of the rectal swabs collected up to 20 days after the cessation of feeding the contaminated feed were positive for Salmonella. The pigs were exposed to at least 21 serotypes in this feed. No harmful effect was noticed in the pigs which corresponded to a previous finding by the author that a 12% isolation rate was found in healthy pigs at slaughter. The author concluded that the main danger of feeding such contaminated feed to pigs was the risk of exposure to humans through meat from these animals contaminated by alimentary contents at slaughter, and that this risk may be ameliorated by withdrawing, before slaughter, feed supplements likely to be contaminated, as none of the pigs became permanent fecal excreters. No Salmonella were found in the pigs' feces after they were removed from the contaminated feed.

A British survey of fecal samples from pigs originating from 344 farms over a two year period, found an isolation rate of 0 to 12 % (Heard et al., 1969). The serotypes isolated had been frequently found previously in animal feeds and the authors suggested that "new infections occur from time to time on the farms via the feed."

In a series of papers, Kampelmacher and co-workers in the Netherlands (Kampelmacher et al., 1965; Guinee et al., 1965; Kampelmacher et al., 1963) examined the incidence of Salmonella in pigs at slaughter, the effect of transport on such incidence, the correlation between Salmonella isolated during life and at slaughter, and the effect of rearing pigs on decontaminated feed. They concluded that piglets may become infected at a very early age ("piglet infection"), possibly from the sow, and that Salmonella persists in the mesenteric lymph nodes without being shed in the feces. They stated that feeding decontaminated feed might suppress a Salmonella infection acquired early in life.

They found that in pigs not transported before slaughter, Salmonella isolations from the feces before and after slaughter were the same and that duration of transportation did not cause an increase in fecal contamination, however, the number of positive fecal samples was larger after transportation. They found that 214 of 566 (37.8%) of normal slaughter pigs were positive for Salmonella.

An increase in isolation rates from cecal contents and rectal swabs after slaughter was found compared to that obtained from rectal swabs before slaughter, although they stated that a single fecal examination was of low value in assessing Salmonella status. They concluded that contamination via the feed, while allowing for the possibility of organisms multiplying in the trough and contaminated by rats at night, appeared to be the means by which apparently normal pigs at slaughter acquired Salmonella infections.

Williams and Newell (1967, 1968) cultured pigs before a four hour transport, after placing in a holding pen, and after 12 to 19 hour holding periods. The Salmonella serotypes in the feed being fed to the pigs was known. Only one of 491 rectal swabs taken before transport was positive for Salmonella. After transport up to 72% were positive, but that number fell to 0-6% after holding for 12-19 hours. They concluded that excretion of feed source Salmonella stopped completely during the overnight holding time and that the numbers of environmental types decreased sharply. The authors postulated that the stresses of transport, handling, crowding, cold, and lack of feed or
water may have acted to trigger a non-excreting pig into becoming an excreter of the *Salmonella* organisms. Stress could have caused an evacuation of the caecum and rapid passage of fecal material, which might have accounted for the higher isolation rate after transport. As the pig adapted to the holding pen, stress was decreased and the numbers of *Salmonella* in the feces abated. They concluded that *Salmonella* from the slaughterhouse environment could infect pigs which would then excrete the organisms for a short time. These organisms could subsequently be demonstrated at slaughter thereby becoming a potential source of carcass contamination. The authors stated that "the primary source of contamination (at slaughter) is most probably the *Salmonella*-excreting pig which has consumed contaminated feed ingredients on its farm of origin."

Williams and Newell (1970) cultured pigs before and after transporting them in a truck for about four hours. All the rectal swabs taken from the pigs in their pens were negative, but after the transport, 6/20 or 30% were positive for *Salmonella*. *Salmonella* was also isolated from the truck which had previously been steam cleaned and disinfected. As *Salmonella* had been isolated from the feed ingredients, the authors concluded that feed source infection occurred on the farm but was not measurable in the undisturbed animal, and may be at numbers less than the infecting dose for the animals.

Niven (1961) stated that the serotypes isolated from animal by-products were not found to correlate with those of *Salmonella* isolated from animals consuming feed containing those by-products. Neither was there a high incidence in the feces of such animals, although at slaughter there was a high degree of *Salmonella* infection in the colon. However, in a study by Lee et al. (1972) in Great Britain, infection found at slaughter was thought to originate on the farm where fishmeal in the feed introduced and maintained the infection in a group of pigs. The same serotypes were found in the fishmeal, and in the pig feces on the farm and in the cecal contents of the pigs at slaughter.

Katsube et al. (1973) studied the distribution of *Salmonella* in the intestinal tract and lymph nodes of the pig and concluded that the cecum was the most important site to culture to detect carriers of the organism.

A Minnesota study (Tay et al., 1989) comparing isolation rates for *Salmonella* in the lymph nodes and cecal contents of pigs at slaughter reported that of 167 (84%) sows that were positive for *Salmonella* at slaughter, *Salmonella* were isolated from 131 mesenteric lymph nodes (66%) and 60 cecal contents (30%). Nine different serotypes were identified. The authors concluded that the *Salmonella* from lymph nodes and other tissues may represent past infection, rather than contamination at slaughter, and that cecal *Salmonella* are more likely related to farm exposure than to infection during transport and holding. The isolation from mesenteric lymph nodes of *Salmonella* from 54% of clinically normal pigs led to the opinion that incising these nodes at slaughter could contaminate the carcass.

**Surveys of Animal Feed and Ingredients for *Salmonella***

Over the years, surveys have been conducted which reflect the occurrence of *Salmonella* in animal feeds and ingredients. Some early findings are listed in Table 1. Those surveys which contained information more particularly on swine feeds are discussed below.

An extensive survey by the USDA (Morehouse & Wedman, 1961) involving 5,712 samples of animal by-products and complete feeds in 31 states found that 718 or 12.57% were positive for *Salmonella*. Of these, complete feeds composed 1415 samples of which 71 or 15% were positive. About the same time, workers in Canada (Isa et al., 1963), sampled 281 feeds and feed ingredients and found 42 or 15% to be contaminated with *Salmonella*. Of 33 samples of pig feeds obtained, one was positive. While allowing that the significance of this incidence rate of *Salmonella* in feeds
was speculative, they concluded that production of feed ingredients free of *Salmonella* would appear to be possible.

A USDA survey in 1966 sought to determine the incidence of *Salmonella* in three of the most common finished feeds and their four major ingredients (Allred et al., 1967). A total of 12,770 samples were collected at feed mills in 26 states. Of 1567 samples of swine feed, 3.13 +/- 0.58% were positive for *Salmonella*. They indicated that "a reduction in *Salmonella* contamination of animal by-product ingredients and the pelleting of finished feeds would be the logical approach to lowering the *Salmonella* contamination rate in swine and poultry feed," while allowing that "feed transmission is only one of many modes of transmitting salmonellosis in the animal population."

Patterson (1972) in Northern Ireland conducted a survey of feeds and feed ingredients of animal and vegetable origin. Of 53 samples of pig meals and pellets 3 or 5.7% were positive. These 3 were unpelleted feed. He stated that a clear link exists between the feeding of contaminated feed and infection in livestock consuming the feed.

In 1993, the FDA conducted a survey of animal and plant protein processors and found that 56.4% of the animal and 36% of the vegetable protein products were positive (McChesney, et al, 1995). A report on an on-going survey of finished feed and primary meal ingredients by the FDA presented the results for 66 meals and 62 complete feeds. Sixteen percent of the complete feeds and 48% of the meals were positive for *Salmonella*. Of the animal meals, 82% were positive and 37% of the meals of vegetable origin. For the swine feeds, 3 of 15 samples (20%) were positive (McChesney, 1995).

An on-farm survey of swine feed and feed ingredients collected from 30 farms in eight states showed that *Salmonella* were isolated from at least one feed or feed ingredients in 14 (47%) of the 30 farms surveyed. Of a total of 1264 samples, 36 (2.9%) were positive for *Salmonella*. Finding *Salmonella* in the feed had a statistically significant association with six herd characteristics surveyed, including lack of bird-proofing, using finisher feed prepared on the farm versus purchasing such feed, and housing pigs in facilities other than confinement in the growing, finishing, gestation, and breeding stages of production (Harris et al., 1996).
Table 1: Early surveys on *Salmonella* in animal feed and ingredients

<table>
<thead>
<tr>
<th>Sample type</th>
<th>No. serotypes</th>
<th>No. pos./total</th>
<th>%</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>fishmeal</td>
<td>NA</td>
<td>5/40</td>
<td>12.5</td>
<td>Bischoff (1955)</td>
</tr>
<tr>
<td>fish, meat meal</td>
<td>22</td>
<td>NA</td>
<td>15.6</td>
<td>Bischoff and Rhode (1956)</td>
</tr>
<tr>
<td>fishmeal</td>
<td>11</td>
<td>9/16</td>
<td>56</td>
<td>Boring (1958)</td>
</tr>
<tr>
<td>vegetable conc.</td>
<td>17</td>
<td>42/910</td>
<td>4.4</td>
<td>Hauge and Bovre (1958)</td>
</tr>
<tr>
<td>animal by-products</td>
<td>28</td>
<td>37/200</td>
<td>18.5</td>
<td>Watkins et al. (1959)</td>
</tr>
<tr>
<td>meat scraps and feed ingredients</td>
<td>41</td>
<td>156/666</td>
<td>23.4</td>
<td>Pomeroy and Grady (1960)</td>
</tr>
<tr>
<td>finished feeds</td>
<td>44</td>
<td>9</td>
<td>British Public Health Service (1961)</td>
<td></td>
</tr>
<tr>
<td>pelleted</td>
<td>ingredients</td>
<td>2.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>cottonseed meal</td>
<td>NA</td>
<td>7/136</td>
<td>5.1</td>
<td>Grumbles and Flowers (1961)</td>
</tr>
<tr>
<td>animal feed</td>
<td>59</td>
<td>718/5712</td>
<td>12.6</td>
<td>Morehouse and Wedman (1961)</td>
</tr>
<tr>
<td>commercial feed</td>
<td>71</td>
<td>3/23</td>
<td>13</td>
<td>Niven (1961)</td>
</tr>
<tr>
<td>by-products</td>
<td>43</td>
<td>75/980</td>
<td>17.9</td>
<td>Pomeroy and Grady (1961)</td>
</tr>
<tr>
<td>animal feeds ingredients, renderings</td>
<td>NA</td>
<td>NA</td>
<td>23</td>
<td>Schotts et al. (1961)</td>
</tr>
<tr>
<td>by-products</td>
<td>NA</td>
<td>NA</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>feed and constituents</td>
<td>10</td>
<td>56/436</td>
<td>12.8</td>
<td>Burr and Helmbolt (1962)</td>
</tr>
<tr>
<td>meat meal</td>
<td>68</td>
<td>178/206</td>
<td>86</td>
<td>Isa et al. (1963)</td>
</tr>
<tr>
<td>feather meal</td>
<td>21/37</td>
<td>57</td>
<td></td>
<td>Williams et al. (1969)</td>
</tr>
<tr>
<td>fish meal</td>
<td>12/68</td>
<td>18</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NA = Not available
Control of Salmonellosis in Swine Feed

The USDA launched a *Salmonella* surveillance program in 1963, gathering information on the numbers and locations of isolations, sources and serotypes in order to increase understanding of the epidemiology of the organism. A report evaluating this program after 15 years concluded that "...the major source of the *Salmonella* problem in man derives from foods of animal origin especially poultry, beef, and pork. Repeated infections of animals occur on the farm in large part because of contamination of feed. Infection persists in some animals, and transmission occurs when animals are shipped to processing plants and are awaiting slaughter. Foods are often contaminated from carcasses and certain organs, especially intestinal contents and lymph nodes" (Gangarosa, 1978).

In 1969, the USDA (USDA Committee on *Salmonella*, 1969) issued a comprehensive report on *Salmonella* which included an overview of control measures for animal salmonellosis. The recommendations at that time were as follows:

- minimize *Salmonella* contamination of animal, poultry, and fish by-products intended for animal feed.
- extend the effort to all feed ingredients and blended feeds.
- emphasize the need for improving feeding and management programs at the producer level, e.g., control of free-flying birds, rodents, and other pests.

For swine producers:

- improve husbandry practices including use of concrete slabs for feeding and pen sanitation.
- design swine buildings and equipment so they can be easily cleaned and sanitized.
- develop breeding herds free of *Salmonella* and other specific pathogens.

Prior to slaughter:

- transportation vehicles must be designed so they can be cleaned and disinfected between uses.
- holding pens at local and terminal markets should be so constructed that they can be easily cleaned and disinfected.*Salmonella* contamination of sources of feed and water should be minimized.

Because of the concern that food-producing animals most frequently became infected with *Salmonella* through the consumption of contaminated feed, a survey for *Salmonella* in animal feeds was done in 1966 (Allred, et al. 1967). Based on these findings (of 1567 samples of swine feed, 49 (3.1%) were positive), a cooperative State-Federal program for the rendering and fish industry was created. The purpose was to reduce the level of *Salmonella* in those animal protein products by identification of areas of contamination and institution of control programs on a voluntary basis. This program was terminated in 1972.

Each participating plant had developed a *Salmonella* control program and it was felt further progress would require authority by federal officials to enforce recommendations. Another reason given was "evidence was still lacking that would indicate the elimination of *Salmonella* from animals feed would have a significant impact on *Salmonella* contamination of red meat and poultry and on the incidence of the disease in man." They concluded that efforts to reduce zoonotic salmonellosis would be to improve sanitary conditions in slaughter plants, educate food handlers and consumers, and conduct more research to minimize bacterial contamination during and after slaughter (Wilson, 1978). The number one research goal of the future according to the 1978 National Salmonellosis Seminar was the need for an in-depth study of the best methods to produce and maintain *Salmonella*-free feeds (Williams, 1978).
Six years later, the International Symposium on *Salmonella* was summarized by the statement that it was time to take what is already known about *Salmonella* and, together with any new information in progress, implement the control measures and the available technologies demonstrated to reduce *Salmonella* contamination of animals and the food produced from them (Mussman, 1984).

**Animal Feeds and the Risk Potential for Human Salmonellosis**

Some workers have indicated that control might depend on the serotypes of *Salmonella* involved. "Studies of *Salmonella* outbreaks have demonstrated that in animals and humans, contaminated food and feed-stuffs of animal origin can be the source of infection. Researchers have suggested that the impact of these agents on human foodborne *Salmonella* infections should be decreased by controlling the twelve serotypes most frequently associated with human infection and disease, rather than to decrease the number of all *Salmonella* that can contact humans" (Snoeyenbos and Pomeroy, 1984).

A review by Newell and Williams (1971) on the control of *Salmonella* regarding feed concludes with the statement that *Salmonella* contamination of pig feeds occurred worldwide and was found most frequently in feed ingredients made from animal, poultry, or fish sources. They emphasized that along with sanitation and pest control, protein concentrates should be *Salmonella* free in order to control this organism on the farm.

Walker (1957) postulated that organic fertilizers made from human sludge, meat and bone meals, hoof and horn meal, blood meal, and fish meal contaminated with *Salmonella* might be responsible for indirect infection of humans through animals and vegetables. He found that 50 of 123 such samples (40%) were found to harbor *Salmonella*.

Williams (1975) reviewed the environmental aspects of salmonellosis and stated that "a great deal of evidence existed which indicates that *Salmonella* serotypes have a path of infection from animal feed to pigs to pork and then to man.

**Salmonella Control in Feed and Ingredient Processing**

Proper terminal heating of meat meal was found to eliminate *Salmonella* (Nape & Murphy, 1971). The primary source of recontamination within the rendering plant was stated to be dust (Hansen et al., 1962). Recontamination by rodents was considered to be the single most important factor (Wedman, 1961).

Recontamination after processing was concluded to be the source of *Salmonella* contamination in one study (Morehouse and Wedman, 1961). At that time the authors concluded that there was not definitive evidence to link organisms from by-products in feeds to specific field occurrences of salmonellosis but that this potential disease threat deserved further analysis. They believed recontamination of animal by-products by rodents, in particular, was the most important single factor accounting for the presence of *Salmonella*. Wild birds, dogs, other animals, humans and contamination from the re-use of feed sacks were also implicated. Fifty percent of environmental samples around rendering plants were contaminated with *Salmonella* and that finished rendered products become contaminated from the environment during the latter stages of processing (Magwood et al., 1965).

Vanderwal (1979) in the Netherlands experimented with decontamination procedures for feed. He found that pelleting under steam with proper temperature and moisture conditions would decontaminate feed as well as the addition of 0.9% formic acid. There was a reduced level of contamination of animals at slaughter and also improved weight gain and feed conversion with either method.
Edel and Kampelmacher (1976) in the Netherlands examined 7756 pigs over a one year period and found 22.3% to be positive for *Salmonella*. Farms which fed pelleted feed instead of meal showed fewer animals to be positive for *Salmonella* at slaughter (20.75 versus 23.7% and 12.9% versus 21%); and fewer serotypes were found on farms with pellet feeding.

Pelleting of finished feed resulted in a 80-90% reduction in mean counts of *Salmonella* due to the heat treatment involved (British Public Health Laboratory Service, 1961).

In England, Ghosh (1972) found that serotypes found in pigs on the farm and in the processing plant corresponded to those isolated from feed. Heat treated pelleting prevented the introduction of *Salmonella* to the pigs as demonstrated by the absence of new serotypes during a two year period.

In a survey of two integrated broiler firms, 60% of the meat and bone samples taken at feed mills were contaminated with *Salmonella*, and 35% of the mash feed samples were positive (Jones et al., 1991). Pelleting the feed was found to reduce the isolation rates by 82%.

In a study comparing the incidence rates of *Salmonella* in pigs and animal feeds in Denmark and England, the much lower rate found in Denmark was attributed to the requirement that imported and domestic feed ingredients of animal origin be sterilized. A narrower range of serotypes was also found in Denmark. This sterilization of animal origin ingredients was thought to reduce pig infections in Denmark with *Salmonella* serotypes other than *S. typhimurium*. (Skovgaard and Nielsen, 1972).

In a study in which an isolated new turkey breeding premise was maintained *Salmonella* free for four years, it was found that when eight isolates of *Salmonella* were subsequently isolated from the premises, five of them were first isolated from finished feed. The authors concluded that feed contamination was a primary source of infection for the herd (Zecca et al., 1977).

McCapes et al. (1991) concluded that the critical control points for feed production were:

- purchase *Salmonella*-free feed ingredients, maintain strict sanitary protocols for personnel, the mill environment, equipment, and transportation
- pasteurize feed after all ingredients have been mixed together
- exercise strict sanitary measures afterwards to protect the feed from becoming recontaminated

Chemical additives for incorporating into complete rations have been studied for many years, particularly for poultry feeds (Westerfield et al., 1970). A diet containing 0.25% formic acid resulted in the elimination of *Salmonella* carriage by growing chicks (Hinton et al., 1985). A mixture of formic and propionic acids in the feed before contamination with *Salmonella* prevented the establishment of infection in chicks (Hinton and Linton, 1988).

Formic acid (0.5%) was found to reduce the rate of *Salmonella* isolations in hen feed and also decrease the incidence of infection in newly hatched chicks (Humphrey and Lanning, 1988). These organic acids, propionic, formic and acetic, have been added to both finished feeds and feed ingredients. They were found to reduce the number of viable *Salmonella* in feed thereby controlling initial contamination and preventing recontamination during processing and transport. This reduction, however, might not be sufficient to decrease the number of viable organisms below the minimum infective dose for some *Salmonella*. They also may not have the expected bactericidal activity in heavily contaminated feed (Sesti, 1994). A Canadian Feed Industry Association (CFIA) brochure recommended the addition of 4% propionic acid to single ingredients and mixed feeds. After testing for freedom from *Salmonella*, the ingredient or feed should be mixed or diluted so that the level of propionic acid did not exceed 0.5% (Blackman et
The addition of Salmonella inhibitors to feeds and ingredients was considered an adjunct to a Salmonella control program and not a substitution (Garland, 1994).

Acidity of the feed was thought to be a factor according to van Schie and Overgoor (1987) who found that Salmonella occurred in a lower percentage of farms which used whey as part of the feed mix (40%) than on farms using only water (80%). The number of samples which were positive for Salmonella on the farms using whey was 19.4% as opposed to 64.1% on the farms using water.

Gamma radiation also has been tried successfully to sterilize animal feeds (Snoeyenbos and Pomeroy, 1984)

Other Factors Regarding Salmonella Control in Feeds

Salmonella has been isolated from a number of animal species, including reptiles and insects, and the environment. In one study, wild animals, birds, and rodents were considered to be potential reservoirs for infection, but that the more probable scenario was that they were infected by the same means as the pigs; the feed (Newell and Williams, 1971).

Salmonella has been isolated from lesser meal worms, American cockroaches, and German cockroaches, which were considered to be mechanical carriers of the organism (Jones et al., 1991). Greenberg (1964) found that Salmonella persisted in flies from the maggot to the adult stages, and contaminated flies may transmit the organism for a distance of at least three miles.

Of 2103 environmental samples and 715 mice and rats collected from 10 poultry farms, 5.1% and 16.2% respectively were cultured positive for Salmonella enteritidis. Salmonella enteritidis was reported to persist in an infected mouse population for at least 10 months (Henzler & Opitz 1992).

A report from Yoshimura et al. (1980) examined Salmonella isolated from animal feed ingredients for their antibiotic sensitivity. Of 110 strains of 41 serotypes, the proportion of resistant strains was 1.8% and no correlation was found between the serotype and the antibiogram of any of the strains. They concluded that Salmonella encountered in feed ingredients may not always originate from animals, and that Salmonella in feed hardly play a role in the emergence of antibiotic resistance in strains of animal origin.

The seasonal incidence of Salmonella infection was reported by Currier et al. (1985), who found that the isolation of Salmonella from cecal contents of 874 pigs at slaughter in Texas did not vary with season, however, more different types of serotypes were isolated during the hot, dry summer and fall seasons as compared to the cooler, wetter, winter and spring season.

Occurrence and Control of Salmonella

Many other countries have reported on the occurrence of Salmonella in animal feedstuffs, and employ various means for its control.

Salmonella control has been on-going in Sweden since 1961. The frequency of occurrence of Salmonella in animals and feedstuffs has been reported every fifth year since 1949. All Swedish feed producing plants are checked for the presence of Salmonella in ingredients, complete feeds and dust samples (Malmqvist, 1995).

Their control program encompassed the following strategies:

- prevent Salmonella contamination in the production chain
- monitor critical points of this chain to avoid contamination with *Salmonella*
- motivate producers to participate by economic incentives
- ensure cooperation and compliance through legal means

Today, meat and poultry produced in Sweden are claimed to be *Salmonella*-free (Wierup, 1991).

Since 1976, Japan has conducted annual surveys of animal feed ingredients. In the period from 1976 to 1982, the incidence of *Salmonella* isolations has varied from 11 to 26% (Sato, 1984).

The *Salmonella* control program in Denmark involved mandatory testing of feed for the presence of *Salmonella* in all plants producing animal feed. Finished products as well as samples taken during production are analyzed, and those batches found to be contaminated must be re-sterilized (Bager et al., 1994). The most recent report available from Denmark indicated that only 0.8% of the 2330 samples of pig feed tested were positive for *Salmonella* (Zoonose-Nyt, 1995).

Serological screening using an LPS mix ELISA test has been adopted as a means to monitor Danish swine herds for the presence of *Salmonella* (Nielsen et al., 1995). Slaughter plants are monitored by the bacteriological sampling of fresh pork for the presence of *Salmonella*. The level of *Salmonella* contamination in fresh pork was reduced from 3% to 1% with the implementation of the control program.

A Dutch survey of poultry feeds and feed ingredients for 1990-1991 reported that 10% of the samples taken were positive for *Salmonella*, and that mash feeds were more frequently (21%) contaminated than pelleted feed (14%). They found that the serotypes isolated most frequently from the feed were not the same as those isolated from the flocks (Veldman et al., 1995).

Some feed and ingredient manufacturers in the U.S. have active programs for the reduction of *Salmonella* in their products. For 20 years the Menhaden fishmeal industry and the National Marine Fisheries Service have participated in a federal inspection program for fishmeal products (Committee on Feed Safety, USAHA, 1994). The National Grain and Feed Association (NGFA) has published quality assurance programs for their members as has the Animal Protein Producers Industry (APPI), which has 235 rendering plants participating in a *Salmonella* testing program. The number of plants participating is reported to be increasing for this voluntary program (Feedstuffs, 1995).

In 1990, the Center for Veterinary Medicine announced a goal of *Salmonella*-free feed and feed ingredients. Progress toward this goal was to be achieved initially by developing and implementing Hazard Analysis Critical Control Point (HACCP) plans for all areas of the feed industry (Mitchell and McChesney, 1991). As application of HACCP programs evolved, it was found that addressing those processing steps for which a safety hazard has not been identified or cannot eliminate or reduce the hazard were best addressed with a prerequisite program approach combined with HACCP (McChesney, 1995). These prerequisite programs would have application to those areas of feed manufacturing such as sanitation, and post-processing handling of product.

**Summary**

As food producing animals are ultimately consumed by humans, we must be concerned about what food the animals consume. The many years of research that have been devoted to dealing with *Salmonella* in animal production point to the fact that there is no quick easy way to eliminate contact by our food producing animals with this ubiquitous organism in their environment. *Salmonellae* occur in many places and are generally very hardy. Control efforts must encompass all those ecological niches from where *Salmonella* might arise in order to be effective. Progress can be made as has been shown particularly in Sweden and Denmark. Implementation of the research findings which exist concerning effective management and control measures must be
encouraged. Ultimately, control of Salmonella will be an on-going long-term process requiring the dedicated efforts of all those involved in animal production for human food.

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