Swine can become infected with PRRS virus through intranasal, intramuscular, oral, and vaginal routes of transmission.

After infection, pigs can shed the virus for as little as a few weeks to as long as several months. While PRRS virus infection can persist in pigs for many months in some situations, the existence of a permanent carrier status in pigs has not been confirmed.

Pigs that become persistently infected with PRRS virus are the most important reason for failure in control and eradication efforts.

In persistently infected pigs, the virus is most likely found in lymph tissues (tonsils, lymph nodes).

The degree to which an infection persists is dependent on numerous factors including the age of the pig at the time of infection, innate immunity of the pig, and characteristics of the specific virus strain.

PRRS virus does not appear to remain viable in the normal environment for more than a few days. Temperature, moisture, the presence of organic matter, and pH all impact the length of time it can remain infective.

Standard cleaning and disinfection protocols should be effective in controlling PRRS virus in the environment.
PRRS Virus – What Happens After a Pig Becomes Infected with PRRS Virus?

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Introduction

Much research has been done exploring what happens to a pig after it becomes infected with PRRS virus. This chapter aims to describe the potential means by which a pig can become infected as well as a discussion about how one pig can pass the virus to another pig. These topics are critical to the development of PRRS control and eradication programs, and new vaccine development.

Routes of Exposure

Swine are susceptible to PRRS virus by several routes of exposure, including intranasal, intramuscular, oral, and vaginal. By either intranasal or intramuscular routes, the minimum infectious dose is low and young swine are readily infected by exposure to 10 or fewer PRRS virus particles (Yoon et al., 1999).

Infection by oral exposure has been demonstrated experimentally. Hypothetically, infection in the field could occur through oral exposure to virus-contaminated feed or water, but it has not been documented. Dispensing vaccine via drinking water offers significant labor-saving advantages over vaccination of individual animals, but anecdotal reports indicate that attempts to date have given negative results. This suggests that the minimum infectious dose by oral exposure is much higher than by intranasal or injection routes. Even so, the potential for infection by oral exposure to PRRS virus-contaminated imported pig meat has become a trade issue.

Outbreaks apparently associated with the use of artificial insemination led investigators to consider the transmission of virus in semen (Robertson, 1992). Shortly thereafter, infection was demonstrated in females following artificial insemination with undiluted semen from PRRS virus-infected boars (Yaeger et al., 1993), extended semen from infected boars (Gradil et al., 1996, Swenson et al., 1995a), and semen to which PRRS virus was added (Prieto et al., 1997a, Swenson et al. 1995a). In one field study, transmission via semen was reported as second in importance only to the introduction of infected pigs as a source of virus (Le Potier et al., 1997).

Virus Shedding

Routes of shedding

Infection of susceptible animals results in the shedding of virus in saliva, nasal secretions, urine, semen, and perhaps feces, with shedding occurring simultaneously from many sites at low levels or perhaps intermittently. Pregnant susceptible females inoculated in late gestation have been shown to shed virus in mammary secretions (Wagstrom et al. 2001).

Shedding of PRRS virus in semen was proven early on (Swenson and Zimmerman, 1993, Swenson et al., 1994a). The period of shedding varies widely among boars (Christopher-Hennings et al., 1996). Swenson et al. (1994a) found infectious virus in the semen of experimentally infected boars for up to 43 days following exposure. Using a nested reverse transcription-polymerase chain reaction, Christopher-Hennings et al. (1995a) detected viral RNA in the semen of experimentally infected boars for up to 92 days post exposure and isolated PRRS virus from the bulbo-urethral gland of a boar euthanized 101 days after inoculation. Frequently, clinical signs in acutely infected boars are mild and transient (Christopher-Hennings, 2001). Feitsma et al. (1992) observed PRRS virus infection in approximately 230 boars, of which about 25 percent showed clinical signs, including poor appetite, fever, and, in some cases, loss of libido. Most boars recovered within one week. Therefore, clinical signs are not an accurate diagnostic measure of PRRS virus infection in boars. Intermittent shedding of virus in semen can occur. Thus, neither negative polymerase chain reaction (PCR) and/or negative virus isolation (VI) results on serum samples nor specific serum antibody levels (S/P values) are reliable indicators of the absence of semen shedding (Christopher-Hennings et al., 1995a, Christopher-Hennings et al., 1996, Christopher-Hennings, 2000).

Voicu et al. (1994) were the first to suggest that PRRS virus might be shed in milk and colostrum, thereby serving as a means of transmission in endemically infected herds. Hypothetically, shedding of virus in milk and colostrum was a possible explanation for the failure of early weaning protocols to predictably eliminate PRRS virus (Clark et al.,
1994, Fangman et al., 1996, Senn et al., 1998). Wagstrom et al. (2001) showed that exposure of susceptible gilts to MLV vaccine or field virus between days 85 to 97 of gestation resulted in the shedding of virus in mammary secretions in the subsequent lactation. Overall, the data suggested that susceptible dams exposed to virus during late gestation shed virus in mammary secretions, but prior immunity inhibited the likelihood of shedding.

The characteristics of fecal shedding of PRRS virus remain unresolved. Yoon et al. (1993) reported extensive fecal shedding by young pigs over a 35-day observation period. In contrast, Rossow et al. (1994) found only 2 positives among 120 samples collected over a period of 28 days after inoculation. Christianson et al. (1993) reported recovery of virus from fecal swabs through day 9 (12 positives among 56 samples) from sows experimentally inoculated with isolate VR-2332, but the investigators raised the possibility that blood contamination of fecal materials may have resulted in the presence of virus in feces. Wills et al. (1997b) did not isolate virus from 36 fecal samples collected from 6 pigs over a 42 day period after challenge. Traditionally, producers expose animals to feces from infected animals in order to infect animals with pathogens known to exist in the herd in order to improve herd immunity and prevent clinical outbreaks. In the case of PRRS virus, the data suggest that fecal feedback will not consistently accomplish this goal. Separate from the question of the presence or absence of PRRS virus in feces, but relevant to the issue, Pirtle and Beran (1996) reported rapid inactivation of virus in fecal slurry.

**Persistent PRRS virus infection**

PRRS virus produces a chronic, persistent infection in pigs. Virus replicates in susceptible cells of infected individuals for several months, thereby resulting in clinically inapparent carrier animals. This is the single most significant epidemiological feature of PRRS virus infection. It profoundly affects all efforts at prevention and control of the disease.

Persistent PRRS virus infection has been extensively documented through transmission experiments and by detection of virus in persistently infected animals. Within a year of the first published report of the identification of the virus, Zimmerman et al. (1992) had reported transmission of PRRS virus from a sow infected 99 days earlier to susceptible sentinels. Following *in utero* exposure at day 90 of gestation, Benfield et al. (2000b) isolated virus from tonsil and lymph nodes from pigs for up to 132 days after farrowing. Wills et al. (1997c) reported isolation of virus from one of four pigs at 157 days post inoculation. Many additional studies have been completed to further characterize the persistence of PRRS virus in different ages and classifications of pigs.

Allende et al. (2000) aptly described PRRS virus persistence as a "smoldering" infection in which the virus is present at lower levels in a continuously decreasing percentage of recovering animals over time. Overall, the data show that persistent infection is a reflection of the ability of the virus to evade the immune system and not a function of pig age at the time of infection. The mechanism(s) by which the virus is able to persevere in the face of an active immune response is not known.

Detection of carriers is problematic. Under experimental conditions in which animals were followed for up to 105 days post inoculation, Horter et al. (2002) reported no significant difference in the antibody response of carrier versus non-carrier animals. That is, it was not possible to predict carrier status based on the enzyme-linked immunosorbent assay (ELISA) serological test response. In the field, Kleiboeker et al. (2002) reported that oral scraping samples from 54 of 191 sows in one herd were PCR-positive. All serum samples from the 54 PCR-positive animals were both PCR and VI negative. Disturbingly, 9 of the 54 were serum antibody (ELISA) negative both at the time of sampling and 4 weeks earlier. In a second herd, 11 of 56 oral scraping samples from sows were PCR positive and 4 of the 11 were VI positive, as well. Again, all serum samples from the 11 PCR-positive animals were both PCR and VI negative. Two of the 11 animals were also serum antibody (ELISA) negative. Although the virus is known to persist in lymphoid tissue, particularly the tonsil, after it is no longer detectable by PCR or VI in serum, the tonsil is not a convenient *ante mortem* diagnostic sample to collect from adult animals. Thus, practical, accurate, and cost-effective diagnostic techniques for the identification of persistently infected pigs are lacking.

Ultimately, control of PRRS must be implemented at the population level. Precise estimates of the percentage of animals in a population that remain persistently infected over time, and the virus loads they carry, are needed. In addition, we need estimates of the probability of transmission between carrier and susceptible animals and the circumstances under which transmission occurs.
Factors of undetermined significance in virus shedding and persistence

Hypothetically, several factors could alter virus shedding and persistence patterns and, thereby, affect the epidemiology of PRRS virus by changing transmission parameters. The most obvious of these is immunity from prior exposure to the virus and is discussed in another chapter. With the exception of prior immunity, none of these factors discussed below has actually been proven to affect either the rate or duration of shedding or persistence.

Differences among virus isolates - Differences in virulence among virus isolates is associated with higher levels of virus circulating within the pig. Halbur et al. (1996) reported that significantly more virus was present in the lungs, lymph nodes, and tonsils of pigs infected with higher virulence isolates as compared to lower virulence isolates. Haynes et al. (1997) found that more tissues were positive in pigs infected with a high-virulence isolate (VR-2385) versus a low-virulence isolate (VR-2431) at 10 and 21 DPI. These data suggest the possibility that isolates that are more virulent might be shed at higher levels for a longer period but other research seems to contradict these findings.

Age of pig at time of infection - Direct comparisons of the effect of age on virus replication in the pig are nearly non-existent in the literature. As discussed above, age has no apparent effect on virus persistence. In one of the few studies examining the age effect, Rosso et al. (1994) found no differences in the duration of detectable virus in the bloodstream (viremia) or virus shedding among 1-, 4-, and 10-week old pigs. However, the general perception is that viremia resolves more quickly in adult versus young animals and other studies confirm this.

Stress - The effect of stress on shedding and transmission by persistently infected animals is unclear but probably of minor importance. Some research has been done in this area and has reinforced this notion.

Bacterial or viral co-infections - Although data is sparse, the available information does not support the hypothesis that co-infections, through direct or indirect effects on macrophages, affect either the level or duration of PRRS virus replication in pigs.

Diet - The impact of a few specific dietary factors on PRRS virus have been studied under controlled experimental conditions, but none have been tested on a broad scale in the field. If present, effects have been limited to the acute phase of the infection. Early in the PRRS pandemic, Bane and Hall (1990) hypothesized a link between to dietary exposure of swine to fumonisin, an immunosuppressive mycotoxin, and "mystery swine disease." A case-control study conducted in mid-1990 found a statistically significant association (p = 0.017) between fumonisin contamination of feed and the risk of "mystery swine disease " (Bane et al., 1992). Farms with > 20 parts per million (ppm) of fumonisin contamination in the feed were at a significantly higher risk (OR=11.2, p = 0.037) and, the risk of "mystery swine disease" increased as the level of fumonisin in the feed increased. Information corroborating an interaction between fumonisin and PRRS virus infection has not been forthcoming.

Host genetic factors - Innate host resistance to disease is an area of strong interest because of the possibility of breeding disease-resistant livestock. To date, this potential has been exploited extensively by poultry breeders and to a much lesser extent by swine breeders. The data on innate host resistance to PRRS virus, as measured by replication of virus within the pig, is sparse. In a small study, Christopher-Hennings et al. (2001) compared the presence of virus in serum, semen, or peripheral blood mononuclear cells over time in adult Hampshire (n = 3), Yorkshire (n = 3), and Landrace (n = 2) boars inoculated with a PRRS virus field isolate (SD-23983). The small sample size and the variation in response among boars precluded the possibility of detecting statistically significant differences among breeds. Halbur et al. (1998) infected Duroc, Hampshire, and Meishan pigs with PRRS virus (VR-2385) at 22 to 38 days of age and compared the lesions 10 DPI. Hampshire pigs had significantly more severe lung lesions than Duroc or Meishan pigs. Meishan pigs had significantly less PRRS virus detected in the lungs, but significantly more Meishan pigs had heart and brain lesions. Durocs had significantly lower serum antibody titers against PRRS virus. The investigators concluded that the differences observed could, in part, be influenced by breed genetics.

Virus Stability in the Environment

Shedding of virus in saliva, urine, and perhaps feces, results in environmental contamination and creates the potential for transmission via fomites. ("Fomites" are defined as inanimate objects that convey infection because they have become contaminated with the infectious agent.) PRRS virus is a fragile virus that is quickly inactivated by drying, however, it can remain infectious for an extended time under specific
conditions of temperature, moisture, and pH. Benfield et al. (1992) examined the effect of temperature on the inactivation of virus isolate VR-2332 suspended in laboratory medium (minimum essential medium) and found that virus infectivity was reduced by 50 percent after incubation for 12 hours at 37° C (99° F). The virus was completely inactivated after 48 hours at 37° C or 45 minutes at 56° C (133° F). Infectivity was unchanged after one month at 4° C (39° F) or 4 months at -70° C (-94° F). Bloemraad et al. (1994) reported the inactivation of PRRS virus under various conditions of temperature and pH as measured by virus half-life. A half-life is the time required for the virus population to decline by one-half. Measuring inactivation as half-lives, rather than absolute numbers, makes comparisons of different treatments and experiments easy. The calculation of half-lives and half-life confidence intervals is described elsewhere (Bryan et al., 1990). As shown in Table 1, under the conditions of the study, inactivation of virus was highly dependent upon both temperature and pH.

Pirtle and Beran (1996) studied the stability of PRRS virus in or on 16 fomites, including plastic, stainless steel, rubber, alfalfa, wood shavings, straw, corn, swine starter feed, denim cloth, phosphate buffered saline, well water, city water, swine saliva, urine, and fecal slurry. At 25° to 27° C (77° to 81° F), infectious virus was not detected on fomites beyond day zero. However, infectious virus was detected in phosphate buffered saline through day 3, well water through day 8, and city water through day 11.

**Disinfection**

Effective disinfection first requires removal of all organic material. Thereafter, infectious agents are inactivated in a temperature- and contact time-dependent fashion specific to the agent and the disinfectant. At "room temperature," Shirai et al. (2000) reported complete inactivation of PRRS virus with chlorine (0.03%) in 10 minutes, iodine (0.0075%) in one minute, and a quaternary ammonium compound (0.0063%) in one minute. The effects of temperature or pH were not explored. Given that PRRS virus is relatively fragile in the environment (Pirtle and Beran, 1996), standard protocols for cleaning and disinfecting facilities should be effective in the control of PRRS virus.

**Summary**

PRRS virus is found in most areas of the world. Within infected countries, 60 to 80 percent of herds (prevalence) are typically infected. Estimates of prevalence are confounded by the use of MLV vaccines in most parts of the world. MLV vaccines have been available since 1994 and antibodies against vaccine virus are not easily differentiated from antibodies against PRRS virus field strains. Population density has a marked effect on the prevalence of PRRS within herds and regions. Even within the same area, larger herds tend to have higher in-herd prevalence than smaller herds.

Swine are susceptible to PRRS virus by several routes of exposure, including intranasal, intramuscular, oral, and vaginal. Exposure to 10 or fewer PRRS virus particles by intranasal and intramuscular routes results in infection (Yoon et al., 1999). Benfield et al. (2000a) determined that a PRRS virus concentration of 2 x 10^3 TCID_50 per 50 ml of semen was sufficient to infect females through artificial insemination. The minimum infectious dose by oral exposure has not been established.

Infection of susceptible animals results in the shedding of virus in saliva, nasal secretions, urine, semen, and perhaps feces, with shedding occurring simultaneously from many sites at low levels or perhaps intermittently. Pregnant susceptible females inoculated in late gestation have been shown to shed virus in mammary secretions (Wagstrom et al. 2001). The infection is a chronic, persistent infection whereby virus replicates in susceptible cells of infected individuals for several months. Shedding of PRRS virus in secretions and excretions results in environmental contamination and creates the potential for transmission via fomites. The virus is relatively labile in the environment and is quickly inactivated by drying, but it can remain infectious for an extended time under specific conditions of temperature, moisture, and pH. Dee et al. (2002a, 2002b) illustrated that PRRS virus could be moved extensively on fomites in the field under winter conditions, i.e., below 0° C (32° F), but to a much lesser degree during warm weather, i.e., 10° to 16° C (50° to 61° F). Standard disinfection and sanitation procedures are effective against the virus, but they must be correctly applied.

PRRS virus transmission most commonly occurs by direct transmission, i.e., close contact between animals or by exposure to contaminated body fluids (semen, virus-tainted blood, or perhaps mammary secretions). The behavior associated with establishing a social order within a group that involves slashes or bites in the shoulders, neck, and head and results in the exchange of blood and saliva and transmission of PRRS virus. Indirect transmission by fomites, vectors, or aerosols may also occur. Of these, transmission via instruments
and medications contaminated with body fluids from PRRS virus-infected animals is the most important. This includes instruments used for ear notching, tail docking, teeth clipping, or tattooing, as well as needles, syringes, medications, and vaccines. Recent research has shown that flies and mosquitoes are capable of mechanical transmission of PRRS virus under experimental conditions (Otake et al., 2002c, 2002d). Aerosol transmission is still an open question. Results of pig-to-pig aerosol transmission experiments are mixed and essential information, e.g., the quantity of virus excreted by pigs and the rate of inactivation of aerosolized virus, is missing.

The ability of PRRS virus to establish carrier animals is the primary challenge to prevention and control. Establishing and maintaining herd immunity in the face of persistent infection is problematic because vaccines that induce long-term protective immunity against different PRRSV isolates and eliminate or reduce virus shedding are not yet available. Finally, if elimination is achieved, herds are vulnerable to re-infection with PRRS virus through the introduction of carrier animals or by area spread. This scenario is reminiscent of other infectious agents, i.e., classical swine fever virus (hog cholera) or African swine fever, which have been successfully controlled and/or eliminated in the past through coordinated regional efforts.

References


Table 1: Estimated half-life of PRRS virus under various conditions of pH and temperature

<table>
<thead>
<tr>
<th>Temperature</th>
<th>4°C (39°F)</th>
<th>21°C (70°F)</th>
<th>37°C (99°F)</th>
<th>56°C (133°F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH 5.00</td>
<td>18.8 hours</td>
<td>—</td>
<td>0.7 hours</td>
<td>—</td>
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<tr>
<td>pH 5.25</td>
<td>—</td>
<td>—</td>
<td>0.6 hours</td>
<td>—</td>
</tr>
<tr>
<td>pH 5.50</td>
<td>—</td>
<td>—</td>
<td>3.1 hours</td>
<td>—</td>
</tr>
<tr>
<td>pH 5.75</td>
<td>—</td>
<td>—</td>
<td>5.7 hours</td>
<td>—</td>
</tr>
<tr>
<td>pH 6.00</td>
<td>—</td>
<td>—</td>
<td>6.5 hours</td>
<td>—</td>
</tr>
<tr>
<td>pH 6.25</td>
<td>50.0 hours</td>
<td>—</td>
<td>4.1 hours</td>
<td>—</td>
</tr>
<tr>
<td>pH 6.50</td>
<td>—</td>
<td>—</td>
<td>2.9 hours</td>
<td>—</td>
</tr>
<tr>
<td>pH 7.00</td>
<td>—</td>
<td>—</td>
<td>2.4 hours</td>
<td>—</td>
</tr>
<tr>
<td>pH 7.50</td>
<td>139.0 hours</td>
<td>20 hours</td>
<td>1.4/3.0 hours</td>
<td>6 minutes</td>
</tr>
<tr>
<td>pH 7.75</td>
<td>—</td>
<td>—</td>
<td>1.4 hours</td>
<td>—</td>
</tr>
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<td>pH 8.00</td>
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<td>pH 8.50</td>
<td>33.3 hours</td>
<td>—</td>
<td>1.3 hours</td>
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</tr>
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</table>

1Table adapted from Bloemraad et al., 1994
2Both half-life estimates reported in Bloemraad et al., 1994


