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# Timeline of infection and detection of PRRSV in the boar

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## Detection of PRRSV infection in the boar and in semen

PRRSV can infect boars and be transmitted through semen causing seroconversion of sows or gilts and infection of embryos, ovaries, and other tissues (Yaeger et al., 1993; Swenson et al., 1995; Prieto et al., 1997). Additionally, boars that are infected with PRRSV may be carriers of the virus and transmission could occur by direct boar contact. To prevent transmission, it is important to know the timeline of infection in the boar and in semen. The methods of detection of PRRSV in the boar are similar to methods used for other swine. Serum can be used for virus isolation (VI) or the polymerase chain reaction (PCR). However, due to the cytotoxicity of semen on cells used for culture, the identification of PRRSV in semen is more consistently observed by using PCR (Christopher-Hennings et al., 1995a). The "swine bioassay" was the first procedure used to identify PRRSV in semen. It consists of injecting semen intraperitoneally (IP) into PRRSV naïve four to eight week old piglets. Serum is then collected from the piglets once weekly for five weeks. If seroconversion occurs, then the semen that was injected into the piglet is considered to have contained PRRSV (Swenson et al., 1994b). This method allows detection of actively replicating PRRSV, whereas PCR may also detect non-viable virus. However, when a comparison was made between the swine bioassay and PCR results, a 94% correlation was observed, which would indicate that PCR, in most cases, is identifying live virus (Christopher-Hennings et al., 1995b).

When semen is used for PCR, it is important to optimize the sample for the best sensitivity. Studies have shown that PRRSV resides within macrophages within the semen. Since studies have indicated that macrophages are the only cell type to support PRRSV replication in vitro, other than continuous cell lines such as MARC-145 or MA-104 cells, it would seem logical that macrophages in semen may be the primary carrier of PRRSV. PRRSV was identified by immunohistochemistry in macrophages in semen samples from vasectomized boars (Christopher-Hennings et al., 1998). Using five vasectomized boars, it was determined that PRRSV was in the cell fraction of the semen in 52 of 52 (100%) of PRRSV positive semen samples, whereas none of the cell-free (seminal plasma)

fractions alone were positive for PRRSV. Seven of the 57 PRRSV positive samples (14%) were positive in both the seminal plasma and cell-free fractions. Therefore, in a small percentage of semen samples, if the cell fraction is PRRSV positive, the seminal plasma fraction may also be PRRSV positive (Christopher-Hennings et al., 1998). It was also found that some semen samples could be misidentified as PRRSV-negative if only whole semen was used (Christopher-Hennings et al., 1995a; 1995b). Another study found that PRRSV could be identified by in situ hybridization in immature sperm cells (spermatocytes) and multi-nucleated giant cells, but not in mature spermatozoa (Sur et al., 1997). Also, using fractionated semen, PRRSV was found in seminal plasma, nonsperm cells, and whole semen, but not in the sperm head (Shin et al., 1997). These studies indicated that mature spermatozoa and seminal plasma are not the primary carriers of PRRSV in semen. Immature sperm cells have been found to have PRRSV antigen within them. However, macrophages alone have been shown to carry PRRSV in semen from vasectomized boars. Therefore, to achieve the greatest sensitivity for the detection of PRRSV in semen, the cell fraction of the semen is used (Christopher-Hennings et al., 1995a; 1995b).

On post mortem examination, tissues can be collected for PRRSV identification and used with either VI or PCR. For antemortem diagnosis using tissues, a tonsil biopsy or scraping may be beneficial for identifying PRRSV, as has been demonstrated with younger swine (Horter et al., 2002; Allende et al., 2000).

## Timeline of PRRSV infection in the boar

The timeline of PRRSV infection in the boar can be divided into early, middle, and late stages of infection based on the duration of experimental studies which have evaluated these time periods from initial infection through 131 days post infection (DPI). There are few controlled studies that have evaluated the diagnosis of PRRSV in boars beyond 100 DPI. Therefore, the stages of infection have been defined as initial infection through 8 days post infection (early), 9 to 35 DPI (middle), and after 35 DPI (late). These time periods are also found to correspond to the humoral response of the boar, since no antibodies are identified in the early period, ELISA and IFA values be-

come positive around 9-14 DPI (middle period) and neutralizing antibodies typically are identified at approximately 35 DPI (late period). A description of these periods follows along with answers to common questions regarding these stages of infection.

#### **Early stage of infection (initial infection through 8 DPI)**

The most consistent finding during this time period is that boars have circulating virus in their blood (viremia).

*If a PRRSV naïve boar is infected with PRRSV, how many hours will it take before the virus can be detected?*

A serum sample can be obtained within a day after infection and VI can identify infectious PRRSV. No studies using adult boars have determined whether this time period is a few hours after infection or a day, but it is known that virus in younger pigs can be found within 12 hours after infection (Rossow et al., 1995). Boars have been found to be viremic by the first day after PRRSV inoculation.

*Can PRRSV be found earlier in serum or semen?*

There is one study which collected serum and semen at 1 DPI (Christopher-Hennings et al., 1995b). All of these boars (four of four) were found to have PRRSV in serum before it was detected in semen. There are other experimental studies in which PRRSV was also identified in serum earlier than in semen (Christopher-Hennings et al., 1997; 2001). Therefore, studies have demonstrated that PRRSV is found in serum earlier than in semen. Pathogenesis studies performed in younger swine have determined that PRRSV is characterized by an initial viremia with subsequent virus distribution to other organs (Rossow et al., 1995). A suggested mechanism for PRRSV entry into boar semen has also been proposed (Christopher-Hennings et al., 1998). It describes an initial viremia with subsequent distribution to monocytes and tissue macrophages. PRRSV replication could then occur in reproductive tissue macrophages or in non-reproductive lymphoid tissue with blood stream trafficking and, finally, PRRSV shedding in semen. This proposed mechanism suggests that PRRSV trafficking to semen may occur later than the initial viremia. However, due to the limited number of studies, this does not preclude the possibility that PRRSV could be found in semen at the same time it is initially found in serum.

*Are clinical signs observed in boars infected with PRRSV?*

During several experimental PRRSV challenge studies in boars, there were few—if any—clinical signs (personal observation). No clinical signs were observed and rectal temperatures were normal throughout a 70 DPI period (Prieto et al., 1996). If clinical signs are observed in boars, they most likely will be detected during the early stages of infection. These signs may include fever, depression,

and anorexia (Swenson et al., 1994a; Christopher-Hennings et al. 1995b; Yaeger et al., 1993) and may only occur for a couple of days (Legeay et al., 1997).

*What is the duration of shedding of PRRSV in semen if a PRRSV vaccine is given?*

Even though the administration of a modified-live vaccine is an extra-label use in adult boars, it is given to attempt to limit shedding of PRRSV in semen from subsequent PRRSV exposures. Typically, the vaccine is shed in semen within the early to middle period of infection. In one study, modified-live vaccine was detected in semen of four of five vaccinated boars between 7-14 days post vaccination (DPV). The fifth boar shed the vaccine between 7-39 DPV (Christopher-Hennings et al., 1997). Using the same vaccine in a second study, vaccine virus was shed in the semen of three boars between days 6-15, 9-12, and 15-21 DPV (Shin et al., 1997). Both studies used PCR to detect the virus in semen. A third study used the swine bioassay in detector piglets to determine whether vaccine virus was shed in semen (Nielsen et al., 1997). At least one of five boars had vaccine virus within the semen at 14 DPV. This was determined since four of five piglets kept in the same room, each injected IP with semen from an individual boar, became PRRSV seropositive. So it may have been that more than one of five boars actually was shedding the vaccine in semen at 14 DPV. By using the swine bioassay for detection, this study also established that the vaccine virus is actively replicating and may be transmitted to other swine. Therefore, in general, the modified-live vaccine is shed in semen, but is most typically shed sporadically during the first two weeks after vaccination. However, a range of shedding has been observed from as short a duration as one day only at 7 DPV or for several days through 39 DPV.

#### **Middle stage of infection (9 to 35 DPI)**

During this period, boars become PRRSV seropositive. In addition to serum antibodies, antibodies in semen have been identified beginning one to four weeks after infection (Oleksiewicz et al., 2001).

PRRSV has been identified in multiple tissue sites of boars by either PCR or VI at 21 DPI. These sites include lymphoid tissues, reproductive tissues, and other tissues including lung, heart, liver, kidney, brain, and small intestine (ileum) (Christopher-Hennings et al., 1998). Most tissues positive by VI were lymphoid tissues, although PRRSV was found by PCR throughout multiple organs. Other studies using younger pigs have also observed localization of PRRSV in multiple tissues at 14 DPI (Sur et al., 1996) and 21 DPI (Rossow et al., 1996; Lawson et al., 1997).

Generally, during this time period, viremia is not as consistently observed as during the early phase of PRRSV infection. In one study by 21 DPI, only three of seven

boars were PRRSV positive in serum by PCR and none of the serum samples were positive by VI (Christopher-Hennings et al., 1998). Other studies have detected infectious virus in serum through seven or nine DPI (Shin et al., 1997; Christopher-Hennings et al., 1995b); 14 or 21 DPI (Nielsen et al., 1997); 10 or 17 DPI (Christopher-Hennings et al., 1997); 21 or 28 DPI (Legeay et al., 1997), and through 14 DPI (Oleksiewicz et al., 2001). Younger pigs may be viremic for eight weeks, but viremia is usually shorter in adult swine (Horter et al., 2002; Bierk et al., 2001; Rossow et al., 1994). However, infectious virus in serum was found to cease in one group of boars between 30 and 58 DPI (Sur et al., 1997).

The average duration of PRRSV shedding in semen generally falls within this middle time period. In a group of 15 boars infected with different PRRSV field isolates, the mean shedding time was 35 DPI with a median of 28 DPI and a range from 4 DPI to 92 DPI (Christopher-Hennings et al., 1995a; 1995b; 1997; 2001). Variability in the duration of shedding from as short as 4 DPI to as long as 70 DPI was also seen after giving the same virus and dose to individual boars at the same time (Christopher-Hennings et al., 2001).

*Is semen quality affected by PRRSV during the early, middle, or late stages of infection?*

Experimental studies have been performed where semen quality parameters were measured and semen abnormalities were not consistently found. There are two studies that followed boars through 21 and 56 DPI that did not observe any semen quality changes (Swenson et al., 1994a; 1994b). However, one study found a transient decrease in semen volume, while concentration, color, motility, and sperm morphology were normal (Yaeger et al., 1993). Another study observed decreased sperm motility (Prieto et al. 1996) or increased numbers of proximal and distal cytoplasmic droplets at 35 and 42 DPI (Prieto et al., 1996). In a field study, it was reported that semen abnormalities were observed approximately 28 days after personnel knew they had PRRSV (personal communication). It is known that fever may affect spermatogenesis, so that semen abnormalities may be seen due to increased rectal temperatures in PRRSV infected boars. However, if the temperature effect is primarily on immature sperm cells in the testis with subsequent abnormalities in mature sperm, this may take approximately 50 days to be observed. This is due to the time period of sperm maturation (Roberts et al., 1994). Therefore, semen abnormalities may not always occur in PRRSV infected boars. However, if they do, they are most likely observed during the middle to late stages of infection and are not usually an early indicator of PRRSV infection.

**Late stage of infection (after 35 DPI)**

This stage is primarily characterized by boars that have recently developed neutralizing antibodies and are not viremic but may still have virus within specific tissue types, particularly lymphoid tissues.

*If a boar is PRRSV seropositive and serum and semen are PRRSV negative based on VI and/or PCR results, will this boar still be infectious to other swine?*

In one study, boars were euthanized and tissues collected after two to three weeks of obtaining PRRSV negative serum, semen, and peripheral blood mononuclear cells (PBMC) (Christopher-Hennings et al., 2001). Seven of eight of these boars still had tissues which were positive by PCR or VI. Tonsil was the only tissue that was positive by VI and was observed in boars euthanized at 40 (two boars) and 47 (one boar) DPI. Very few reproductive tissues compared to lymphoid tissues were positive by either VI or PCR between 40-88 DPI. In a study using PRRSV infected, aviremic sows, it was demonstrated that virus could still be transmitted to 3 of 12 contact sows at 49, 56, and 86 DPI (Bierk et al., 2001). Therefore, it might be assumed that boars with tissues that are still PRRSV positive by PCR or VI may still be infectious during this time. However, no transmission studies during this time period have been specifically performed using boars.

*How can you decrease the risk of obtaining PRRSV-positive semen from a PRRSV seropositive boar?*

Since an average shedding time of PRRSV within semen is estimated to be 35 DPI (n = 15), this phase may also be characterized by boars that may not be shedding PRRSV in semen. In a field study using PRRSV seropositive boars, a total of 131 boars had semen tested by PCR for the presence of PRRSV (Eisenhart and Brown, 1998). Boars tested had been in quarantine for 25 days or greater and 12 of 131 (9.1%) were found to have PRRSV in their semen. When the quarantine period was extended to 45 days or greater and a second group of 440 boars were tested, 7 of the 440 (1.6%) were found to be PRRSV positive in semen. It is not known when these boars were initially exposed to PRRSV; however, this study shows that few boars actually harbor PRRSV in semen, in spite of being PRRSV seropositive. Additionally, the extended quarantine period of 45 days or greater may be beneficial in preventing PRRSV-contaminated semen from being disseminated.

*How do you detect PRRSV-infected animals if serum, semen, and PBMCs are PRRSV-negative?*

If the boar is PRRSV-seropositive, it is difficult to determine whether the boar is still harboring PRRSV in spite of PRRSV-negative semen, serum, or PBMCs. It is easier to determine PRRSV infection during the early stage, rather than during the late stage of infection. Therefore, establishing a PRRSV-naïve herd has some advantages diagnostically. As has been seen in other studies, the use

of sentinel animals and/or tonsillar scrapings may be useful in detecting persistently infected animals.

### Timeline summary

In summary, it should be noted that there are few studies that use large numbers of boars for evaluation. This is often times due to the high cost of analysis and logistics in obtaining semen over time from a large numbers of boars. Therefore, some of these conclusions are obtained from limited analysis. However, there are certain trends among adult swine that confirm studies describing PRRSV infection in boars. Trends that have been observed concerning the timeline of infection in boars include the following:

- During the early stages of PRRSV infection (up to 9 DPI), boars are viremic, PRRSV seronegative, and will shed the virus in semen at some point during this time period.
- During the middle stages of PRRSV infection (9 through 35 DPI) viremia will usually disappear, boars will become PRRSV seropositive, and the average shedding time of PRRSV in semen will occur in this time period (mean = 35 DPI, median = 28 DPI, n = 15). However, variability in the duration of PRRSV shedding in individual boars may be as short as 4 DPI or as long as 92 DPI. Multiple tissues (reproductive and non-reproductive) are also PRRSV positive by VI and PCR.
- During the late stage of PRRSV infection (greater than 35 DPI), neutralizing antibodies are observed. Most boars will be aviremic and PRRSV will be disappearing from semen. Tissue sites most commonly infected include lymphoid tissues, specifically tonsil and lymph nodes. It is unknown whether boars will be able to transmit PRRSV during this period of time. However, even if serum, semen, and PBMCs are PRRSV-negative, the boar may still be harboring PRRSV and has the potential to transmit the virus.

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