SÍNDROME DISGENESICO Y RESPIRATORIO DEL CERDO

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History "Mystery Swine Disease", later renamed Síndrome Disgenésico y Respiratorio del Cerdo or Porcine Reproductive and Respiratory Syndrome (PRRS), was recognized as a new disease of unknown cause in North Carolina USA in 1987. The disease was characterized by abortions and infertility of sudden onset, the birth of weak or dead piglets, and increased mortality in young pigs, commonly as a result of secondary respiratory infections. In most herds, response to treatment was poor, and producers were left with no alternative but to allow the disease to run its course. Typically, clinical signs persisted for 2-to-4 months.

The infectious nature of PRRS became apparent when clinical outbreaks moved rapidly through swine herds located in the North Central region of the United States in 1988. In 1990, PRRS appeared in Germany (Epidemisch Spättabort der Sauen) and, similar to the pattern previously seen in the USA, spread rapidly across Europe.

Terminology The Oficina Internacional de Epizootias (OIE) currently recognizes the terms Síndrome Disgenésico y Respiratorio del Cerdo (Spanish) , Syndrome Dysgénésique et Respiratoire du Porc (French), and Porcine Reproductive and Respiratory Syndrome (English).

Etiology The cause of PRRS was determined in 1991 when Koch's postulates were fulfilled with a previously unrecognized, enveloped, RNA virus (Terpstra et al., 1991; Wensvoort et al., 1991). PRRSV is an Arterivirus, a group of RNA viruses which includes equine arteritis virus, lactate dehydrogenase-elevating virus of mice, and simian hemorrhagic fever virus (Plagemann and Moennig, 1992; Meulenburg et al., 1993; Conzelman et al., 1993). Genomic sequence analysis suggests that PRRSV contains as many as 6 structural proteins, but only 3 structural proteins with molecular masses of approximately 15, 19, and 26 kilodaltons (kd) have been consistently demonstrated (Nelson et al., 1993). Current evidence indicates that the 15 kd protein is a nucleocapsid protein, whereas the 19 and 26 kd proteins are presumed to be components of the viral envelope. The immunobiological roles of the viral proteins is not yet understood, but researchers speculate that the 26 kd protein may be associated with induction of serum neutralizing antibodies against PRRSV (Yoon et al., 1995a).

Antigenic diversity Marked antigenic variations are known to exist among PRRSV isolates. Wensvoort et al. (1992) reported serological differences between Lelystad virus, a prototype European PRRSV, and U.S. PRRSV isolates using polyclonal porcine antibody. Nelson et al. (1993) used a panel of 3 monoclonal antibodies (MAb) against the 15-kd nucleocapsid protein to characterize the reactivity pattern in 57 European and 63 North American PRRSV isolates. These investigators demonstrated that antigenic differences exist between European and North American PRRSV isolates with respect to the nucleocapsid protein. It appeared that isolates could be divided into European or North American subgroups based upon the reactivity patterns with the panel of 3 Mabs. However, several recent studies have demonstrated that both European and North American PRRSV isolates are antigenically more diverse than previously believed (Dea et al., 1996; Drew et al., 1995; Nelson et al., 1996; Yang et al., 1996; Yoon et al., 1995b). Sequence analysis of the viral genome has provided evidence for a genetic basis for the antigenic diversity observed among PRRSV isolates (Katz et al., 1995; Meng et al., 1994; Meng et al., 1995b).

Infection in swine herds Originally, PRRS was characterized as an acute outbreak of reproductive failure in sows, including anorexia, abortions, early farrowings (107-112 days of gestation), increased stillborns, mummies, weak born pigs, and delayed return to estrus. Respiratory distress, fever, interstitial pneumonia, and increased preweaning mortality was present in neonatal pigs. In grower-finishers, infection sometimes resembled swine influenza, or in many cases, no clinical signs were present.

Currently, it is recognized that clinical effects vary widely among infected farms. In many herds, infection is inapparent and productivity is apparently unaffected. Some infected herds report occasional respiratory disease outbreaks in young pigs, or periodic outbreaks of reproductive disease. A few herds experience severe, chronic disease problems, particularly in nursery pigs. In these herds, infections with secondary bacterial pathogens, *Salmonella choleraesuis*, *Streptococcus suis*, or *Haemophilus parasuis* typically occur concurrently with PRRS virus infections. Following the introduction of PRRSV, it is common for herds to go through small outbreaks periodically.

Pathogenesis The pathogenesis of PRRS virus infection is poorly understood. Virus is present in the blood stream as early as 12 hours after inoculation (Benfield et al., 1994), but under experimental conditions, gross lesions are observed only occasionally in the lungs of inoculated pigs. Microscopic lesions may be seen in lung, lymph nodes, heart, and blood vessels.

It is relatively easy to cause reproductive disease with PRRSV in pregnant, PRRSV-naïve animals under experimental conditions. But in contrast to reproductive disease, clinically overt respiratory disease has been difficult to consistently produce in the research environment with PRRSV infection only.

So why does PRRSV infection cause devastating clinical respiratory disease in some herds and not in others? The conventional explanation for the differences in clinical severity among herds is that herds are infected with PRRSV isolates that differ markedly in virulence. But under experimental conditions, virus isolates from clinically devastating outbreaks generally produce few effects when "reasonable" challenge doses are inoculated into conventional pigs housed under research conditions, i.e., healthy pigs housed under optimum conditions of space, hygiene, and stocking density. And so far, researchers have not found a correlation between genotypic sequence variation and postulated differences in isolate virulence (Meng et al, 1995a).

Other factors which are postulated to influence the course and degree of clinical respiratory disease in clinically affected herds include age at time of infection, breed (Halbur et al., 1992), concurrent pathogenic infections (Galina et al., 1994), and the exacerbation of infection by the presence of low levels of PRRSV-specific antibodies, i.e., antibody dependent enhancement (Yoon et al., 1996).

We are currently doing research to understand the interactions between PRRSV and other factors that may interact to produce more severe clinical disease. In our model we have investigated the interactions of PRRSV, *Salmonella choleraesuis*, and stress on young swine. In preliminary studies, 5-week-old segregated, medicated early weaned pigs were randomly assigned to one of 8 treatments consisting of all possible combinations of three factors: *S. choleraesuis* on day 0, PRRSV on day three, and dexamethasone on days 3 to 7. Dexamethasone was used to imitate stress.

As compared to *S. choleraesuis* infection alone, pigs treated with (*S. choleraesuis* + PRRSV) or (PRRSV + *S. choleraesuis* + dexamethasone) grew less, shed *S. choleraesuis* at higher levels and for a longer period of time, had more *S. choleraesuis* in tissues, and showed more severe clinical signs. The results of this study suggest that clinical PRRS may involve interactions among concurrent infections and stressors in the field

Geographic Distribution PRRS virus infection is present in all swine producing areas of the world, including North America, Europe, and Asia (Chang et al., 1993; Correa-Girón et al., 1994; Shin et al., 1993). PRRSV is a relatively new agent in the North American swine population. A retrospective serologic study showed that PRRSV entered the Iowa swine population between 1980 and 1985 (Zimmerman et al., 1997a). None of 1,425 serum samples collected from Iowa swine in 1980 were positive by the indirect fluorescent antibody (IFA) test. Among samples collected in 1985, 3.8% (1/26) of herds sampled were PRRSV-infected. This was followed by an increase in prevalence in each successive year. Among samples collected in 1988, 63.0% (17/27) of herds sampled were positive. In the United States as a whole, a 1990 serologic study involving 87 farms from 18 states found that 82.7% of herds tested were infected (Bautista et al., 1993).

Epidemiology The formulation of strategies for the prevention and control of PRRS virus is dependent upon a thorough understanding of the means by which transmission occurs. At this point in time, however, our knowledge of the transmission of PRRS virus is relatively rudimentary. The following points highlight the current information:

1. PRRS virus is highly infectious; probably more so than most other infectious agents of swine with which we are familiar. The dose required to achieve infection is extremely low. Inoculation of ≤ 10 PRRS virus particles by either intranasal or intramuscular routes is sufficient to infect swine (K-J Yoon, unpublished data).

2. PRRS virus is not highly contagious. Transmission requires relatively close contact between animals. This is in sharp contrast to earlier thought. It was once postulated that aerosolized virus was the primary route of transmission of PRRS virus and aerosol transmission was thought to occur over a distance of up to 20 kilometers. Since PRRS virus is present in the upper respiratory tract and oropharyngeal area of infected pigs, this was not an unreasonable hypothesis. However, Wills et al. (1994) found that transmission among penmates occurred much more readily than transmission across a space of only 18 inches.

3. Infection produces a chronic carrier state. Zimmerman et al. (1992) first reported transmission by direct contract from sows infected 99 days earlier to commingled susceptible animals. Albina et al. (1994) demonstrated transmission of PRRS virus by pigs infected 15 weeks earlier. More recently, isolation of virus from oropharyngeal samples for up to 157 days after experimental inoculation was reported (Wills et al., 1997a). Overall, these data have provided overwhelming evidence that PRRS virus persists in swine for a prolonged period of time. For that reason, clinically healthy carrier animals must be considered an important potential source of virus.

4. Infection results in the shedding of virus in saliva, urine, and semen. Wills et al. (1997b) detected PRRS virus in saliva for up to 42 days and in urine for up to 14 days. Swenson et al. (1994) detected infectious virus in the semen of experimentally infected boars for as long as 43 days following exposure. Using a polymerase chain reaction (RT-PCR), viral RNA has been detected in the semen of experimentally infected boars through day 92 post infection (Christopher-Hennings et al., 1995b). Transmission of PRRS virus to females using artificial insemination with undiluted semen from experimentally infected boars has been demonstrated (Yaeger et al., 1993). Transmission of PRRS virus to females using extended semen from experimentally infected boars has also been shown to occur (Swenson, personal communication).

5. We hypothesize that PRRS virus is transmitted to young pigs in colostrum and milk. In support of this concept, PRRS virus has been recovered from colostrum and milk collected from gilts and sows challenged in the third trimester (85 to 90 days of gestation). In a pilot study, a single milk sample was collected from each of 5 sows and PRRS virus was found in 3 of these samples (Zimmerman, unpublished data). Research to study shedding of PRRS virus in milk and colostrum is currently in progress .

6. In the absence of moisture, PRRS virus is quickly inactivated in the environment. In general, PRRS virus is a fragile virus which is quickly inactivated in the environment. The persistence of PRRS virus on fomites has been studied in, or on, 16 fomites, including plastic, stainless steel, rubber, alfalfa, wood shavings, straw, corn, swine starter feed, denim cloth, phosphate buffered saline, saline G, well water, city water, swine saliva, urine, fecal slurry (Pirtle and Beran, 1996). At 25 to 27 C virus did not persist on fomites beyond day zero, except for phosphate buffered saline through day 3, saline G through day 6, well water through day 8, and city water through day 11. If kept moist and cool, PRRS virus can remain infectious for an extended period of time. At 4° C and under ideal conditions of moisture, the half-life (inactivation of ½ of virus population) is approximately 90 days. At 22° C and identical conditions of moisture, the half-life falls to around 2 days (K-J Yoon, personal communication).

7. Non-porcine host exists, but are they important in transmission? Although rodents are not susceptible to PRRS virus (Hooper et al., 1994), some avian species, mallard ducks in particular, are susceptible to PRRS virus (Zimmerman et al., 1997b). Mallards were exposed to PRRS virus in drinking water and virus isolation was done on feces collected from cages. Recovery of virus from pooled mallard feces up to 25 days after exposure proved that they were susceptible. In a second experiment, mallard-to-mallard transmission was demonstrated by infecting ducks with feces from ducks shedding virus. And finally, swine were shown to be susceptible to mallard-derived virus. That is, pigs intranasally exposed to PRRSV isolated from mallard feces became viremic, seroconverted by ELISA, and transmitted the virus to sentinel swine.

It is not likely that mallards are active participants in the transmission of PRRS virus to swine, although these birds might theoretically serve as a reservoir of new virus types. The more important questions would be, "Are there other susceptible non-porcine species of which we are unaware?" and "Do they play a role in the day-to-day epidemiology of PRRS virus?"

Immune response Development of immunity following infection has not been well characterized. The return of herd reproductive performance parameters to normal levels following a clinical episode of PRRS is evidence that some type of immunity develops following exposure. Protection against subsequent reproductive losses appears to be of long duration in individual animals, at least against homologous virus. Experimentally infected sows were protected against reproductive losses when challenged with homologous virus over 300 days after initial exposure (Zimmerman, unpublished data). Immunity against respiratory disease is poorly understood.

Infected pigs develop a humoral immune response to PRRSV which may be detected by several serological assays (Yoon et al., 1995a). Western immunoblot analysis detected antibody specific for the 15 kd viral protein by 7 days post inoculation (Yoon et al., 1995a). Initial detection of antibodies to the 19, 23, and 26 kd proteins were detected from 9 to 35 days after inoculation. The 26 kd protein, and perhaps the 19 kd protein, were determined to be the most important viral proteins in terms of virus neutralization, as indicated by the disappearance of the virus from serum. An antigen-specific T-cell respone to PRRSV has been shown in pigs following infection (Bautista et al., 1996), but induction of cell-meiated immunity (CMI) and the role of CMI in the control of PRRSV infection in not well understood. Interestingly, PRRSV has recently been shown to persist in the face of an active immune response, as indicated by isolation of virus from the experimentally infected pigs for up to 157 days after challenge (Wills et al., 1997a).

The protection conferred by colostral antibody is presently an area of investigation. Current reports and clinical observations indicate that passive immunity may plays a limited role in preventing infection or decreasing the severity of the disease in young animals.

Diagnosis The diagnosis of PRRSV infection is made through pathological examination; detection of infectious virus, viral antigen, or viral genomic material in clinical specimens; and/or detection of PRRSV-specific antibody.

Pathological examination

Interstitial pneumonia is the most characteristic histological lesion of PRRSV infection (Rossow et al., 1994). Pulmonary lesions are characterized by three main changes:

- thickening of alveolar wall by infiltration of macrophages and lymphocytes;
- type II pneumocyte hypertrophy and hyperplasia; and
- accumulations of necrotic debris and mixed inflammatory cells in alveolar spaces.

Other PRRSV lesions observed less frequently include lymphadenopathy, lymphohistiocytic myocarditis, rhinitis, and encephalitis (Rossow et al., 1994).

Lung, tonsil, lymph nodes, brain, and nasal turbinate are preferred specimens for histopathological examination and immunoassays on tissues.

Virological tests

Virus isolation is the definitive diagnostic test of PRRSV infection. Serum is the preferred specimen for virus isolation because pigs are viremia for a prolonged time (2 to 6 weeks) and PRRSV is more stable in serum than in tissues (Van Alstine et al., 1993). PRRSV can be also isolated from many tissues. Preferred tissues samples include lung, tonsil, and lymph nodes. However, tissue must be fresh if virus isolation is to be successful. Infectivity of PRRSV can be retained for 1 month at 4°C and for at least 18 months at -70°C; however, the virus is rapidly inactivated at 37°C. Consequently, freezing or refrigeration of specimens during transport to a diagnostic laboratory is highly recommended. Recently, lung lavage and recovery of pulmonary macrophages for virus isolation, has been reported to be useful (Mengeling et al, 1996). The practicality of this procedure in live pigs in the field is in question.

To date, PRRSV is known to replicates only in two different types of cells: porcine alveolar macrophages (Wensvoort et al., 1991) and a clone of an African Monkey Kidney cell line (MA-104) identified as MARC-145 (Kim et al., 1993). Porcine alveolar macrophages (PAM) are sometimes reported to be more susceptible to PRRSV than MARC-145, but not all laboratories agree on this point. PAMs are expensive to use and may harbor adventitious agents. In addition, PAMs from different pigs often vary in their susceptibility to PRRSV. For these reasons, MA-104 or MARC-145 offer certain advantages for routine diagnostic use.

The frozen tissue section fluorescent antibody (FA) test and immunohistochemistry (IHC) may be used for detecting PRRSV antigen in tissues. The direct FA test on frozen tissue sections is an inexpensive and rapid test. The test is specific but is not always very sensitive. In particular, tissue quality (e.g., autolysis) affects test results. In contrast, IHC is useful for detecting viral antigen in formalin-fixed tissues. Two types of test have been developed: immunoperoxidase test (Halbur et al., 1994) and immunogold silver staining (Magar et al, 1993). IHC is more sensitive than direct FA examination of frozen tissues, but takes more time and is more expensive than the FA test. For direct FA examination, fresh or frozen tissues should be submitted. Tissues should be fixed in 10% neutral buffered formalin if submitted for IHC detection of PRRSV.

Polymerase chain reaction (PCR) tests (Suarez et al 1994; Christopher-Hennings et al., 1995a) and *in situ* hybridization technique (Larochelle et al., 1996; Sur et al, 1996) have been developed and used for detecting genomic material of PRRSV in clinical specimens. PCR is a highly sensitive test and its primary diagnostic application has been detecting PRRSV RNA in semen of boars and pigs persistently infected with PRRSV (Christopher-Hennings et al., 1995b; Shin et al, 1996).

Further understanding of the molecular biology of PRRSV has made it possible to further characterize PRRSV isolates using a restriction fragment length polymorphism (RFLP) assay. The RFLP assay involves virus isolation followed by PCR amplification of ORF5, then restriction endonuclease digestion, followed by electrophoresis of the products (Wesley et al., 1996). Considering the high rate of mutation of RNA viruses, the utility of this differential test for the long-run is uncertain.

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Serology tests

The indirect fluorescent antibody (IFA) test, serum virus neutralization (SVN) test, immunoperoxidase monolayer assay (IPMA), and enzyme-linked immunosorbent assay (ELISA) have been developed for the detection of antibodies specific for PRRSV. Among those tests, the IFA, SVN and ELISA tests are currently being used in most North American veterinary diagnostic laboratories.

The IFA is thought to have high specificity (99.5%) but unknown sensitivity for individual animals. An advantage of the IFA test compared to ELISA is that the magnitude of the titer can be determined. A titer of 16 or 20, depending upon the initial serum dilution for the test, is considered positive. Since it is subjective in interpretation, the endpoints of IFA titers often vary among technicians and laboratories. Furthermore test results or endpoint titers will vary, depending on how much the PRRSV strain used in the assay differs from the isolate that infected the pig.

An IFA test for the detection of PRRSV-specific IgM antibodies has recently been developed (Park et al., 1995). This test was reported to be useful to detect acute/recent PRRSV infection. A titer of 16 or 20 is considered positive. Importantly, research data demonstrated a high percentage (81%) of isolation of PRRSV from IgM-positive samples. However, false positive results due to the possible nonspecificity of the test have been a concern. This test requires further evaluation and is not currently in routine use.

Several formats of ELISA have been described: indirect ELISA using a sample to positive ratio (S/P) system, an indirect ELISA using direct OD values (Cho et al., 1996), and a blocking ELISA (Houben et al., 1995). With respect to the ELISA kit from IDEXX laboratories Inc., an S/P ratio >= 0.4 is considered positive. The test is reported to be sensitive (100%) and specific (99.5%). Although the magnitude of the S/P ratio might be correlated with the magnitude of IFA titer, the manufacturer does not recommend interpretating the S/P ratio in this way at this time. Many veterinary diagnosticians/practitioners consider a S/P ratio of 2.5 or greater to be indicative of a recent or active infection (G. Erickson and H.T. Hill, personal communications), but this may not hold true for individual animals. Automation which results in less variation and high quality control are considered to be a strong merit of the test. In addition, several other advantages of ELISA are: 1. detection of antibody against both North American and European PRRSV strains; 2. fast turnaround time; and 3. licensure by USDA and AgCanada.

The SVN test is also considered to be a specific test, but previous studies have suggested that the SVN test is less sensitive than the IFA and ELISA tests. Currently, a titer of >=4 is considered positive. A recent report indicated that the sensitivity of the SVN test could be increased by adding fresh normal swine serum to serum being assayed (Yoon et al., 1994). Even so, the SVN test is best used as a research tool rather than a routine diagnostic test.

Interpretation of serology results and considerations

In pigs exposed to PRRSV under experimental conditions, virus-specific antibodies are reported to be first detected by the IgG-IFA, ELISA, and the SVN test at 7-11, 9-13, and 9-28 days PI respectively, and reach their peak value by 30-50, 30-50, and 60-90 days PI, respectively (Yoon et al., 1995). PRRSV specific IgM antibodies are reported to be detected within 5 days PI and persist 21-28 days PI (Park et al 1995). It is generally estimated from experimental and field observations that the IFA, ELISA, and the SVN antibody titers approach undetectable levels by 4-5, 4-10, and 12 months post infection, respectively (Yoon et al., 1995). The same timeframe would be expected in animals vaccinated without a history of previous exposure.

Several problems or limitations should be taken into account when interpreting PRRS serology. Serological information from a single sample is not sufficient for diagnosing clinical PRRS in an individual animals because PRRS virus infection is highly prevalent (80%) in swine herds. Positive results may or may not mean that PRRSV caused clinical disease. Negative PRRS serology on one sample in time have several possible interpretations, as well:

-- the pigs are not infected with PRRSV;
-- the pigs were recently infected with PRRSV and not yet seroconverted;
-- the pigs were infected with PRRSV but has become seronegative; or
-- the test employed was negative because of low sensitivity or laboratory error.

Therefore, if using single point samples, PRRS serology should be used to determine if a herd has been exposed to PRRS virus; not whether individual animals are infected.

Antibodies specific for PRRS virus often do not persist for the lifetime of an animal. The relatively short duration of IFA and/or ELISA antibodies has led to the recommendation that young pigs be tested in order to establish herd PRRSV status. In single-site, farrow-to-finish swine herds, the seroprevalence of PRRSV infection usually highest in the grow-finish unit. Serum from 10 finishing pigs is usually considered to be an adequate sample size to determine whether the herd has been exposed to PRRSV. For multi-site production systems, each stage of production represents a single population, so each site should be sampled.

Diagnosis of PRRSV infection as the cause of reproductive failure or respiratory disease can be achieved by showing seroconversion using paired samples or a change in antibody titer in paired samples. For a more definitive diagnostic evaluation of PRRS with respect to current infection, it is recommended that serological information should be interpreted in combination with results from virus isolation (e.g., viremia).

Using IgM IFA, IgG IFA and SVN tests, 3 categories of serologic profile have been described among pigs in herds exposed to PRRSV: noninfected, acutely infected, and pigs with antibody decay (Dee et al., 1996). Noninfected pigs can be identified as negative by all 3 tests. Acutely infected pigs were defined as pigs with IgM and/or IgG IFA titers of >=64, but no detectable SVN antibody.

Some research data suggest that SVN antibody specific for PRRSV plays a role in clearing the virus from the blood circulation (Yoon et al., 1995; Nelson et al., 1994). However, prolonged viremia and persistent infection of PRRSV in the presence of circulating antibody, as well as antibody dependent enhancement of PRRSV infection by the presence of low concentration of virus-specific antibody, bring the protective role of antibody into question. Consequently, it is important to determine if the presence of neutralizing antibody correlates with protective immunity.

Broad antigenic variation among PRRS virus isolates is a concern in interpreting the serological information because false negative results may be due to the strain of virus in use at a diagnostic laboratory. Such potential problem may be overcome using the commercial ELISA kit (IDEXX Laboratories) because the IDEXX kit contains antigens from several different isolates of PRRS virus. In fact, this kit has been shown to detect antibodies against both North American and European PRRS virus isolates.

Although many limitations and pitfalls exist in PRRS serological, it is still very useful in: 1. monitoring the process of clinical disease; 2. evaluating the PRRSV compatibility of donor and recipient herds; and 3. evaluating vaccine efficacy.

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