REVIEW

PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME (PRRS): A REVIEW, WITH EMPHASIS ON PATHOLOGICAL, VIROLOGICAL AND DIAGNOSTIC ASPECTS

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SUMMARY

Despite early attempts to control the spread of the disease, porcine reproductive and respiratory syndrome (PRRS) has now become endemic in many countries including Britain. The occurrence of subclinical herd infections, the prolonged circulation of virus within herds and probable aerogenic virus spread all mitigated against the success of control measures. The origin of the disease is unknown but the causative agent has been shown to be an arterivirus with shared features to lactate dehydrogenase virus of mice. There is evidence of extreme genetic and antigenic variability between American and European isolates.

PRRS virus has a predilection for alveolar macrophages and does not grow in most cell lines. In infected pigs, viraemia can persist for many weeks in the face of circulating antibodies and little is known about the mechanisms by which immunity to infection develops. A wide spectrum of disease has been reported from the field, accompanied in some cases by heavy economic losses. Reproductive and perinatal losses were most prominent when the disease first appeared. In the endemic phase, PRRS may be more significant as a contributory factor to a post-weaning respiratory syndrome of young pigs of 3–8 weeks. On-farm techniques have been developed to reduce the recycling of PRRS virus from older infected nursery pigs to the younger newly weaned pig. Vaccines are now marketed for the control of PRRS, but are not licensed for use in Britain. Improvements in knowledge of virion composition and antigenic stability and in the nature of the immune response of the pig should result in genetically engineered subunit vaccines becoming available.

Diagnosis of PRRS is still difficult as many animals do not show clinical signs and may only be detected by serology and often only when other respiratory diseases are being investigated. Now that the infection is wide-
spread, serological testing must be properly targeted and interpreted to give meaningful results about virus circulation. An increasing arsenal of diagnostic methods are becoming available to detect virus in both fresh and fixed specimens. The pathogenic mechanisms of PRRS remain poorly defined and more work is needed to reveal the nature of the interaction between PRRS virus and other factors in disease.

INTRODUCTION

Porcine reproductive and respiratory syndrome (PRRS) is a new disease that initially spread as a pandemic (de Jong et al., 1991a; Lindhaus & Lindhaus, 1991; Zimmerman, 1991) but which has become endemic in many countries throughout the world. It appeared in the USA in the 1980s (Dial & Parsons, 1989; Keffaber, 1989) where it was known as mystery swine disease. Since then it has been reported in Canada in 1987, Germany and The Netherlands in 1990, Belgium, Britain and Spain in 1991 and subsequently many other European countries as well as the Far East. The true extent of the spread of the disease certainly is far greater than has been documented. Currently, the disease is on list B of the OIE index of contagious diseases. The disease has also been known by a wide variety of other names including porcine epidemic abortion and respiratory syndrome (PEARS) and in Britain, blue-eared pig disease. The causal agent is a virus first isolated at the Central Veterinary Institute at Lelystad (Pol et al., 1991; Terpstra et al., 1991; Wensvoort et al., 1991).

CLINICAL SIGNS

One of the most obvious features of PRRS disease outbreaks is the variability of clinical effects (Fiedler, 1991; White, 1992), with subclinical infections being common (Robertson, 1992a; Morrison et al., 1992a). Important factors in determining the nature and prevalence of the disease are likely to be pig density, pig movements, air quality, health status, housing systems, quantity of virus present (Robertson, 1991; Dee, 1992) and strain of virus (Halbur et al., 1994a). Severe signs are more likely in large herds, where pigs are purchased, where quarantine is not used and where there are slatted floors (Fielder, 1991; Vogel et al., 1991; Edwards et al., 1992).

All age groups on a farm may be affected so that breeding herds show the widest variety of clinical signs. Systemic signs may include death, especially in the acute phase of infection (Hooper et al., 1992), anorexia, pyrexia, agalactia, lethargy and sometimes skin discolouration. Respiratory signs can include laboured breathing and coughing. All of these signs, but particularly anorexia, progressively move through a herd giving an appearance of a "rolling inappetance" over several days. In sows and piglets the skin lesions may include blue (cyanosed) ears, blue vulvas, blue skin areas or diamonds and erythematous plaques (Fig. 1). The discolouration may be transitory, lasting only a few minutes. It appears to be most common 5–7 days after the clinical appearance of the disease. The acute clinical phase in
Fig. 1. PRRS in a sow: patchy skin discolouration affecting snout, ears neck and abdomen.

the breeding herd may affect 5–50% of the animals (de Jong et al., 1991a; White, 1992).

Reproductive losses are also a feature of the disease and may continue for 4–5 months, occupying an entire reproductive cycle within a herd. Abortions may occur from as early as 22 days post-mating through to 109 days, although premature and late farrowings are much more common. Affected litters may comprise mixtures of stillborn, mummified or decomposing piglets as well as weak and apparently normal newborn piglets. Some newborn piglets shown an unusual doming of the head. Infertility, including delayed returns to oestrus, persistent re-breeding, and persistent anoestrus have been described (Keffaber, 1989; Hoefling, 1990; Gordon, 1992; Hopper et al., 1992), although a large study of German sows revealed no changes in the rate of returns to oestrus or in the farrowing interval (Grosse Beilage & Grosse Beilage, 1993).

Boars may show the same clinical signs as sows (principally pyrexia, anorexia and coughing) but in addition may show loss of libido (Feitsma et al., 1992; Hopper et al., 1992) and a temporary reduction in semen quality (de Jong et al., 1991a; Prieto et al., 1994). Feitsma et al. (1992) have described a reduction in weekly sperm output 4 weeks after clinical signs, with a return to normality by 7 weeks post infection. They also noted a reduction in the motility of sperm and increases in morphological abnormalities, with an increase in rejected ejaculates from 2 to 10% during the clinical phase of the disease. The ejaculates had returned to normal quality by 13 weeks post clinical onset.

Pre-weaning morbidity and mortality is a major feature of the disease. Litters are
often unthrifty, "splay legs" are common and many deaths occur in the first week of life. Laboured breathing may be seen along with conjunctivitis and periorbital oedema. Bleeding disorders are sometimes associated with PRRS in the neonatal period, resulting in navel bleeding and massive bruising at iron injection sites (Hopper et al., 1992). In the USA, an increase in pre-weaning mortality from 3.1% before a PRRS outbreak to an average 7.4% after the outbreak (it reached a peak of 16%) was noted (Stevenson et al., 1993).

The acute phase may last several months but is often followed by a long period of respiratory disease which we have termed "post-weaning respiratory syndrome" and which in the USA has been reported to last as long as 2 years (Loula, 1991). A major component of both pre- and post-weaning illness is the development of secondary infections (Blaha, 1992; White, 1992; Pijoan et al., 1994). A number of viral diseases have been commonly found in association with PRRS, including swine influenza and porcine respiratory coronavirus in Britain, encephalomyocarditis virus in the USA (Carlson, 1992) and paramyxovirus both in the USA (Halbur et al., 1993) and France (Brun et al., 1992). It is not known to what extent these viruses may act synergistically. A whole range of bacterial diseases may increase in prevalence following PRRS (Collins & Rossow, 1993; Zeman et al., 1993) and in the USA these include *Haemophilus parasuis*, *Streptococcus suis*, *Salmonella cholera-suis*, *Actinobacillus pleuropneumoniae*, *Mycoplasma hyopneumoniae* and *Pasteurella multocida*. In Japan, a possible interaction between PRRS and *Mycoplasma hyorhinus* may result in a chronic respiratory disease characterised by laboured breathing, conjunctivitis and stunting (Shimizu et al., 1994).

Reduced thrift may persist throughout the whole finishing period with a poor response to antibiotics or vaccination and with deaths occurring sporadically, weeks or even months after the initial problems in the farrowing rooms. The exact influence of PRRS *per se* on the performance of finishing pigs is difficult to assess. Blaha (1992) reported that in Germany before the epidemic the finishing mortality was 2.2%; 3 months after an epidemic 4.3% and 8 months after 2.8%. We have noted that the frequency of lung lesions may increase from 30% to 70% in pigs born during and after PRRS, and that the annual piglet production may be decreased by 5–20%. However, it is probable that most finishing herds are infected without clinical signs.

Commonly, signs of PRRS are noted in the same sequence as the order of production i.e. sows, piglets, finishers. However, Albina et al. (1994) reported that an influenza-like syndrome in the finishing pigs may precede signs of PRRS in the farrowing rooms. Only occasionally are second waves of clinical infection encountered that are similar to the acute phase of herd infection (de Jong et al., 1991b). Nevertheless, a virus can continue to circulate for prolonged periods in both breeding and finishing herds (Harris et al., 1992; Keффaber et al., 1992; Terpstra et al., 1992; Thacker, 1992; Stevenson et al., 1993) and may cause reproductive wastage in bought in gilts and persistent post-weaning respiratory problems.

**ECONOMIC LOSS**

Losses are extremely variable, both in extent and duration and it is important to discriminate between the effects of the disease in the epidemic and endemic...
phases. In many cases in Britain economic losses have been minimal but others have reported extreme economic loss (Hoefling, 1990; Dykhuizen et al., 1991; Loula, 1991; Vogel et al., 1991; Frankena et al., 1992; Polson et al., 1992; White, 1992) varying between 1–25 pigs and £65 per sow per year (Blackburn, 1991; Mortensen & Madsen, 1992; Brouwer et al., 1994) and 0–20% of annual production (Lindhaus & Lindhaus, 1991; Raymakers, 1991; Van Alstine, 1991; Zimmerman, 1991) or an annual loss in the USA of $18 per finishing space (Moore et al., 1990). An additional burden may be loss of trading status for seropositive breeding stock.

PATHOGENESIS

In the epidemic in Britain, the interval from introduction of infected stock to the first obvious inappetence of the sows, taken as the initial clinical sign, was 14–37 days (Robertson, 1991). In Belgium this was said to be 10–18 days (Varewyck, 1991) and in the USA 3–24 days (Dee, 1992). Most animals become ill 4–5 days after experimental exposure to PRRS virus (Terpstra et al., 1991; Edwards et al., 1992; Christianson et al., 1992; Plana Duran et al., 1992). In the experiments reported by Wensvoort et al. (1991) 6 months old specific pathogen free (SPF) pigs developed illness within 2 days of contact with affected sows. Variable incubation periods may reflect differences in the pathogenicity of different virus strains, but other factors such as dose, route and intensity of observation are also likely to be important. Postnatal challenges to piglets have generally produced rather mild illness, followed by full recovery. Gross pathology has been minimal (Pol et al., 1991; Christianson et al., 1992; Paton et al., 1992a; Rossow et al., 1994), whilst microscopy has usually revealed interstitial pneumonia and in some American studies, encephalitis and myocarditis (Collins et al., 1992). PRRS virus can be isolated from the lung, plasma, serum and blood cells for 5 weeks or more after infection, co-circulating with antibody which is detectable within 2 weeks (Ohlinger et al., 1992b; Paton et al., 1992b; Rossow et al., 1992).

Reproductive signs are usually not evident before 25 days post infection. Several studies report the effects of challenge inoculation of pregnant sows (Terpstra et al., 1991; Christianson et al., 1992; Dea et al., 1992; Plana Duran et al., 1992; Yoon et al., 1992a; Christianson et al., 1993; Albina et al., 1994; Botner et al., 1994) describing various adverse effects on subsequent piglet viability. Infected sows can be viremic within 24 h of infection and this may persist for up to 14 days (Christianson et al., 1992). Transplacental infection may be possible early in pregnancy (Loula, 1991). In utero challenge just after insemination reduced the conception rate compared to controls, although the numbers of animals studied meant that the significance of the finding was uncertain. Those animals that did conceive farrowed uninfected pigs (Lager et al., 1994). Both Christianson et al. (1993) and Mengeling et al. (1994) found that transplacental infection in mid-gestation was inefficient, although foetuses directly inoculated at this time supported virus growth. However, Lager et al. (1994) demonstrated that oronasal exposure of sows at 30 days gestation induced reproductive failure. Although microscopic uterine and placental changes have been detected (Christianson et al., 1992; Stockhofe-
Zurwieden et al., 1993), Vynckier and Pensaert (1993) found no evidence of virus replication in the uteri of sows inoculated per vagina. Since foetal and placental abnormalities have not been consistently found, there is still no adequate explanation of how foetal deaths and reproductive failure are brought about. Differences in virulence may exist between PRRS viruses and in the study of Botner et al. (1994), transplacental virus transmission did not result in increased foetal deaths.

Galina et al. (1994) have confirmed that PRRS does predispose to other infections by producing an experimental model for Streptococcus suis type 2 meningitis. Clinical disease, suppurative meningitis and an abundant growth of S. suis, was only observed when pigs were previously experimentally infected with PRRS virus. Van Reeth et al. (1994) observed potentiation of illness caused by both swine influenza and porcine respiratory coronavirus in pigs experimentally challenged 4 days after infection with PRRS virus. Other experimental attempts to confirm field observations that PRRS acts synergistically with a variety of pathogens have not been conclusive (Pijoan et al., 1994).

PATHOLOGY

The pathological changes in the respiratory tract are suggestive of PRRS but are not pathognomonic. Field cases of PRRS infections without severe secondary bacterial infections are usually grossly normal at autopsy (Done et al., 1992). There may be clear fluid in the thoracic cavity of weak-born piglets and occasionally pulmonary consolidation. In our experience, light microscopical changes have been confined to a mild to severe interstitial pneumonia, with occasional catarrhal pneumonia (Paton & Done, 1992), but more extensive lesions of the respiratory tract, including rhinitis, have been described from the Netherlands (Pol et al., 1991) and the USA (Collins et al., 1992; Rossow et al., 1992). We have not seen in Britain the blood vessel changes described by Goovaerts & Visser (1992), the perivasculitis (Hoefling, 1990; Collins et al., 1992) the mononuclear myocarditis (Hoefling, 1990; Collins et al., 1992), the splenitis with depleted lymphocytes (Pol et al., 1991) or the thymic tonsillar crypt or lymph node depletion described by Pol et al. (1991) and Ohlinger et al. (1991a). Despite attempts to do so, we have also not seen the inflammatory lesions in the placentae from PRRS-infected sows nor demonstrated the virus-like structures in the endothelial cells of foetal and placental capillaries (Stockhofe-Zurwieden et al., 1993). This may be a reflection of the relatively low pathogenicity of PRRS viruses in Britain.

In the USA, the prototype virus ATC-2332 produces interstitial pneumonitis, lympho-mononuclear encephalitis and lymphoid mononuclear myocarditis (Collins et al., 1992) but we have not seen either CNS or heart lesions following naturally acquired or experimentally induced infections with British viruses. We have however, seen ultrastructural changes, with the degeneration of alveolar macrophages and loss of ciliated epithelial cells of the bronchioles with an excessive vacuolation of the endoplasmic reticulum. Lager and Ackerman (1994) are the only authors to report foetal lesions, consisting of focally extensive pulmonary haemorrhage with bronchial bud degeneration and necrosis.

PRRS viral infection causes a startling reduction in the number of alveolar mac-
Rophages collected by bronchoalveolar lavage (Molitor et al., 1992). In normal pigs, alveolar macrophages comprise 90%+ of the cells recovered, but in acute PRRS infection this proportion falls to approximately 50%, with a relative increase in lymphocyte and neutrophils. Following experimental infection with ATC-2332 in the USA, there was a reduction in blood lymphocytes and monocytes, particularly T lymphocytes, within 3 days of infection but the levels recovered to normal by day 14 post-infection (Christianson et al., 1992; Zhou et al., 1992). By 28 days post-infection, leucocyte recovery is complete and there may even be enhanced responsiveness to foreign antigens (Molitor et al., 1992). Increased susceptibility to other pathogens is therefore most likely in the immediate aftermath of infection. High levels of alpha-I acid glycoprotein (an acute phase reactive protein indicative of tissue injury) have also been reported in the sera of young pigs from herds with PRRS (Bane et al., 1992).

TRANSMISSION

Being enveloped, the virus survives relatively poorly in the external environment. Virus can survive for several years in deep frozen tissues, but only 1 month at 4°C, 48 hr at 37°C and less than 45 min at 56°C (Benfield et al., 1992). The half-life for virus survival is decreased at a pH of below 5 or above 7, but live virus can be recovered from carcass meat stored for 48 hr at 4°C (Bloemraad et al., 1994).

Transmission may be by nose to nose contact or by aerosols (Wensvoort et al., 1991; Terpstra et al., 1991) and virus may enter new herds by movement of pigs and probably by airborne spread (Komijn et al., 1991; Robertson, 1992a). Spread within a herd is usually quite rapid and in the first 18 months of the British epidemic, approximately 75% of tested sows had become seropositive within 3 weeks of disease being suspected. Terpstra et al. (1992) showed that approximately 90% of sows seroconverted within 3 months of the virus being introduced into a single closed breeding herd. Urine and faeces have been reported to contain virus (Yoon et al., 1993) although Rossow et al. (1994) found faecal isolation was only occasionally possible. Similarly, virus has been isolated from the semen of experimentally infected boars by one group with relative ease (Swenson et al., 1994), but by others less consistently (Prieto et al., 1994). Epidemiological and experimental data suggest that PRRS can be spread by semen through the use of artificial insemination if semen is collected during the acute stage of infection in the boar (Robertson, 1992b; Yaeger et al., 1993).

PRRS virus is not known to occur in any other reservoir species (Hooper et al., 1994) but a preliminary report by Zimmermann et al. (1993) suggested that migratory fowl can become infected and are therefore possible vectors. The most important method of transmission for nursery and finishing pigs is probably contact with older infected animals (Stevenson et al., 1993, 1994; Dee & Joo, 1994a). Acutely infected animals readily infect other animals by contact up to 14 weeks post infection (Terpstra et al., 1992) and there is an isolated report of contact infection after 99 days (Zimmerman et al., 1992). A recent report suggests that virus may be recovered from the oropharynx of pigs up to 157 days after infection (Wills et al., 1995). Post infection corticosteroid treatment induced a re-excretion
of virus in one study (Albina et al., 1994) but not in another (Terpstra et al., 1992).

At the herd level, virus may or may not continue to circulate depending on the management practices and the herd structure (Keffaber et al., 1992; Freese et al., 1994; Torrison et al., 1994). Even in closed breeding herds, virus may circulate persistently in weaned pigs, infection occurring at around the time when colostral antibodies have been lost at 3–6 weeks of age (Joo & Dee, 1993; Albina et al., 1994; Freese et al., 1994). Large finishing operations purchasing pigs of variable disease and immune status, provide ideal conditions for persistent virus circulation. There is little documented evidence from the field for herd breakdowns resulting from virus excretion by persistently or latently infected individuals (Potter, 1994; Paton & Drew, 1995).

THE VIRUS

Many agents, both viral and bacterial, were considered as potential causes of mystery swine disease, but only the PRRS virus, first isolated by the Dutch, fulfilled Koch’s Postulates (Pol et al., 1991; Terpstra et al., 1991; Wensvoort et al., 1991). Shortly after, the virus was isolated in Germany by Ohlinger et al. (1991b) and in the USA by Collins et al. (1991) and subsequently a related agent was identified in Britain using Dutch methodology (Paton et al., 1992a).

The PRRS virus has a diameter of 45–65 nm containing a 20–35 nm nucleocapsid core (Benfield et al., 1992; Meulenberg et al., 1993a). It passes through a 200 nm filter but is partially trapped by one of 50 nm (Ohlinger et al., 1991b; Benfield et al., 1992) and it has a density of 1.19 g ml⁻¹ in caesium chloride (Ohlinger et al., 1991b; Wensvoort et al., 1991). It is enveloped and therefore sensitive to lipid solvents. The virus has a single positive stranded, polyadenylated RNA genome of approximately 15 kb containing seven open reading frame (ORF) (Meulenberg et al., 1993a), the largest of which, ORF1a and 1b, are thought to encode the viral polymerase. At 15 kDa putative nucleocapsid protein (N) and two putative envelope proteins of 18–19 kDa (M) and 24–26 kDa (E) have been identified (Nelson et al., 1993) and shown to be virion proteins (Bautista et al., 1994; Meulenberg et al., 1995) (Fig. 2). The N protein is encoded by ORF7 and elicits a strong immune response. Anti-N monoclonal antibodies (mAbs) have demonstrated both conserved and variable epitopes (Nelson et al., 1993; Drew et al., 1995). The M protein is encoded by ORF6 and is unglycosylated. It is probably a type III integral membrane protein, as found in coronavirus, and anchored by three successive hydrophobic domains. The E protein is glycosylated and is encoded by ORF5. It possesses a large internal hydrophobic region which could act as a membrane anchor. It is not known whether the products of ORF 2 and 4 are structural proteins, but they have not been identified in virions. A single ORF 3 product specific mAb recognized only a small proportion of PRRS virus isolates, indicating that the expressed protein is antigenically polymorphic (Drew et al., 1995). Reactivity of the mAb with a purified virus preparation suggests that the protein is part of the virion. Kwang et al. (1994) have cloned and expressed ORF4 and reported that only 65% of PRRS seropositive sera reacted with their recombinant protein.
In morphology, genome organization, replication strategy and protein composition, the virus closely resembles lactic dehydrogenase virus (LDV) of mice equine arteritis virus (EAV) and simian haemorrhagic fever virus. Collectively this group have been termed arteriviruses and although currently classified in the Togaviridae on grounds of virion and genome size and icosahedral nucleocapsid symmetry (Benfield et al., 1992; Ohlinger et al., 1992a; Plagemann & Moennig, 1992), the genomic organization and translation strategy suggest a closer link to coronaviruses and toroviruses (Godeny et al., 1993; Meulenberg et al., 1993a, b). By sequence homology, PRRS virus most closely resembles LDV, but the two do not cross-react serologically. Indeed no serological cross-reaction has been detected between PRRS virus and any other virus. All of the arteriviruses infect
macrophages (Plagemann & Moennig, 1992), all are capable of producing long lasting, asymptomatic infections and all demonstrate strain variation.

The origin of PRRS virus is unknown. Strains found in the USA are, for the most part antigenically distinct from those found in Europe, although some serological cross-reaction occurs (Wensvoort et al., 1992; Nelson et al., 1993) and recent investigations suggest that "European-type" strains are also present (Bautista et al., 1993b). Lesser differences are also detectable between different European isolates (Suarez et al., 1994; Drew et al., 1995). Genetic comparisons also indicate considerable differences between American and European isolates including deletions as well as point mutations (Murtaugh, 1993; Kwang et al., 1994; Mardassi et al., 1994a; Meng et al., 1994).

**IMMUNOLOGY**

In herds where PRRS virus persists, sows do not suffer repeated reproductive losses, indicating that some form of protective immunity does develop. Previously infected sows neither transmitted virus to their foetuses nor suffered reproductive failure when rechallenged in late gestation (Gorcyca et al., 1993; Lager et al., 1994).

The protective value of antibody is uncertain and the mechanisms by which virus is cleared are unknown. Co-existence of virus and antibody in serum for several weeks implies that early antibodies are not very effective at clearing virus. Whether or not virus mutates to avoid neutralization is not established. Piglets sucking immune sows appear to be protected from infection (Molitor, 1993), and in herds where virus circulates persistently, young pigs generally become infected after colostral antibodies have been lost (Albina et al., 1994). Albina et al. (1994) also demonstrated that clinical disorders in piglets born to infected sows were at their worst when the piglets had no detectable antibodies. However, although immune colostrum may protect, passively transferred antibodies may not, suggesting a role for cellular immunity (Molitor, 1993). In vitro, virus neutralizing effects of antibody depend on the cells used to culture the virus. In alveolar macrophages, antibody enhances rather than neutralizes infectivity (Choi et al., 1992; Christianson et al., 1993), however, in cell lines, neutralization can be demonstrated (Benfield et al., 1992; Frey et al., 1992; Morrison et al., 1992b). There is very little information concerning cellular immunity to PRRS virus or concerning the extent of cross-protection that may exist between immunity developed against diverse strains of the virus. Some cross-protection has been claimed for an attenuated live virus PRRS vaccine (Gorcyca et al., 1995).

**DIAGNOSIS**

Clinical diagnosis is not always straightforward. Similar respiratory and/or reproductive symptoms can be produced by other viruses such as influenza virus, porcine parvovirus, Aujeszky's disease virus and encephalomyocarditis virus. In addition, the variable severity of PRRS and the modifying effect of different secondary agents greatly complicate recognition of the disease. In some instances,
only close attention to production records (to demonstrate for instance an increase in stillbirths or pre-weaning mortality) will show that there has been infection (Schukken et al., 1992). Random sampling of herds during the early part of the PRRS epidemic in Britain clearly demonstrated that many introductions of the disease go completely unrecognised. Laboratory confirmation of a diagnosis of PRRS is therefore usually prudent and the most valuable methods are serology and virus isolation or detection. Histopathology has been quite useful to identify the pneumatic process, but is only helpful early on in outbreaks, before bacterial pneumonic pathology obscures the relatively mild viral lesions.

Virus has been isolated from a range of tissues including bone marrow, spleen, thymus, tonsils, lymph nodes, heart, brain, liver and kidney. However, serum is a convenient and fairly reliable specimen for sick animals (Van Alstine et al., 1993), whilst foetal fluids or lung can be readily collected post mortem. Mengeling et al. (1995) recommended collection of alveolar macrophages flushed from the airways of freshly killed pigs. Autolysis rapidly inactivates the virus and this probably explains why mummified foetuses are not a good source of virus. If there is reproductive failure, stillborn and weak newborn pigs are a better source. The virus can be grown in porcine alveolar macrophages (PAM) harvested by lung lavage and many strains can also be isolated in continuous monkey kidney cell lines CL2621 and MA104 (Benfield et al., 1992; Kim et al., 1993). Because of individual strain differences in growth on PAM or cell lines, culture on both is the preferred option (Bautista et al., 1993a). The cytopathic effect of PRRS virus for macrophages has been described by Pol et al. (1992) and Paton et al. (1992a). Cellular homeostasis is disturbed and surface projections are lost and eventually virus is released by exocytosis.

Field strains vary greatly in their cultural characteristics, some producing obvious cytopathic effects within 3–4 days of the first passage but others requiring three passages over 3–4 weeks. Non-cytopathic isolates have also been reported and it is therefore necessary to confirm the presence of virus by immunostaining. Indirect immunostaining methods have also been used to detect viral antigens in histological sections of tissues such as lung and spleen (Magar et al., 1993; Halbur et al., 1994b). PCR-based methods of detecting PRRS virus have been developed (Mardassi et al., 1994b; Suarez et al., 1994; Van Woensel et al., 1994) but are not yet routinely applied to clinical specimens.

Serology is the most widely used laboratory diagnostic method and a number of techniques are available. The immunoperoxidase monolayer assay (IPMA) was the initial test developed at Lelystad (Wensvoort et al., 1991) and has been extensively used in Europe. It is based on immunological detection of antibodies specifically bound to fixed PAM cells infected with PRRS virus. A variant of the test widely used in North America, uses immunofluorescence staining (IFA) (Yoon et al., 1992b; Frey et al., 1992), but both give comparable results (Fichtner et al., 1994). Serum antibody can be detected from as early as 6 days after infection but usually within 14 days. Titres usually reach a peak (up to 1:40 000) at 5–6 weeks after infection and antibodies persist for a variable time thereafter, some pigs becoming seronegative after 4–6 months. The requirement for PAM cells and the necessity to read the test microscopically, limits the numbers of sera that can be tested at once and results in a high labour input, and therefore a high cost. Since PRRS is
now widespread, single samples will not indicate whether or not infection has been recent and paired serum samples are to be referred. A sample size of 30 will allow 95% confidence of detecting a 10% or higher seroprevalence (Dea et al., 1992). In finishing herds where there is a higher seroprevalence, fewer samples will be needed and a sample size of 10 will allow 95% confidence of finding herds with a seroprevalence of at least 30% or higher (Morrison et al., 1992c).

The majority of piglets show seroconversion during the nursery period and 3–4 month old pigs are therefore a good indicator of infection (Thacker, 1992). In endemically infected herds, most piglets are seronegative at weaning but 80–100% are seropositive at 8–10 weeks of age (Freese et al., 1994). By contrast, the majority of sows in such herds may be seronegative (Stevenson et al., 1994). Specific antibody is often detectable at birth in prenatally infected animals and such antibody may co-exist with circulating virus. Transplacental infection can therefore be demonstrated by the presence of virus or of specific antibodies in pre-colostral blood and ascitic fluid of stillborn or weak piglets (Terpstra et al., 1991; Christianson et al., 1992).

An indirect ELISA using PAM-derived antigens has been developed by Albina et al. (1992) and is widely used. It is easily automated and therefore relatively cheap. A number of other ELISA formats have also been investigated (Houben et al., 1995) and commercial test-kits are now available.

Virus neutralization by serum can be detected if cell lines are used for virus growth. Neutralizing antibodies develop slowly and the test is not very sensitive compared to IFA and IPMA. Virus strain specificity may also be narrower. However, Yoon et al. (1994) have modified the test and increased its sensitivity so that antibodies can be detected as early as 11 days post infection.

All serological tests may be problematic because of antigenic variation in PRRS (Beilage et al., 1992; Frey et al., 1992; Wensvoort et al., 1992; Bautista et al., 1993b), differences in antibody persistence in individual animals and uncertainty over significance with respect to virological status.

**TREATMENT**

Antibiotics may or may not reduce the effects of secondary bacterial infections (Hopper et al., 1992) and various supportive therapies can improve the survival of neonates. Anti-pyretic agents have been used in pregnant sows (Ahl et al., 1992), but their value is unproven. The level of challenge from infected piglets can be lowered by reducing stocking rates and by taking out ill pigs. Other management procedures that may mitigate losses include delaying the rebreeding of affected sows, using artificial insemination to bolster natural service and delaying iron injection and tail docking of neonates (Ahl et al., 1992).

**PREVENTION AND CONTROL**

Both killed and modified live virus vaccines have become available in some countries and recombinant subunit vaccines are also under development. Plana Duran (1994) described a killed, oil adjuvanted vaccine based on a Spanish isolate
of PRRS virus (Cyblue, Cyanamid) intended for protection against reproductive
disease in gilts and sows. Initial vaccination involved two intramuscular (i.m.)
doses given 21 days apart, booster vaccinations being recommended during sub-
sequent lactations. Experimental challenge of pregnant sows revealed a 70% pro-
tection in terms of pigs born alive and surviving to 7 days. Field studies on the first
500 000 doses used suggested a significant benefit from vaccination in terms of
repeat breeding levels, piglets born per sow per year, percentage of abortions,
numbers of piglets weaned and number of stillbirths. From the USA, Gorczyca et al.
(1995) reported on a live attenuated vaccine (RespPRRS, Boehringer), adminis-
tered by a single i.m. injection. Although vaccination produced a detectable
viraemia, it was shown to be safe in late gestation sows and vaccine virus was appar-
tently not transmitted from vaccinates to susceptible contact pigs. In growing pigs,
the vaccine elicited a protective immunity within 7 days, that lasted at least 16
weeks. Compared to naive animals, challenged vaccinates had a reduced level and
duration of viraemia, and reduced lung lesions and grew better. Field trials sug-
gested that the vaccine offered significant protection to nursery pigs in units with
endemic PRRS virus infection.

A medicated early weaning system has been described by Clark et al. (1994).
Although it is effective against many pathogens (H. parasuis, A. pleuropneumoniae,
M. hypopneumoniae and Aujeszky's disease virus), it did not prevent infected dams
from transmitting PRRS to their progeny. Standard zoosanitary methods, such as
quarantining and serological screening of new stock and restrictions on visitors
may help to keep units free of the disease. If pigs have to be bought in then sero-
logically positive animals should be imported into serologically positive herds and
vice versa. Seronegative replacement gilts can be introduced into seropositive
herds at an earlier age than normal (3–4 months) to allow for viral exposure
before breeding (Dee & Joo, 1994b). If the status is uncertain, quarantine and
exposure to nursery pigs of the importing unit is a suitable policy if replacement
gilts are bought in before they are mated. However, the possibility remains that
infection may be introduced, even into closed units, by airborne virus spread and
taphylographical isolation is the best defence against this. Artificial insemination
cannot, without further precautionary measures, be considered a foolproof method
of introducing genetic diversity without PRRS. Paton and Drew (1995) described
how extended quarantine arrangements could permit seropositive boars to be
introduced into a stud without transmission of PRRS to the resident stock.

As with other respiratory pathogens, all in/all out farrowing and fattening
should help to reduce virus persistence but are not always successful in large air
spaces. In endemically infected breeding herds, weaning batches of pigs “off-site”
to isolated nurseries can break the cycle of infection from older to younger ani-
mals resulting in PRRS infection free fattening (Dee & Joo, 1994a; Christianson et
al., 1994).

CONCLUSION

A great deal has been learnt about the new virus and the complete genome of the
prototype Lelystad virus has been sequenced. Naturally occurring and experimen-
tally induced infections have been documented in detail, laboratory diagnostic methods have been developed and patterns of spread have been studied. Important questions that have not been fully answered include: (1) where did the disease come from? (2) Where precisely does virus replicate in the pig? (3) What are the pathogenetic mechanisms for the respiratory and reproductive losses? (4) Why is there a prolonged viraemia and what is the basis for protective immunity? (5) Does PRRS predispose to other infections and if so how? (6) Why are there such considerable differences between American and European isolates? (7) How important is the virus as a cause of losses in endemically infected herds?

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