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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-

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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 choleraesuis, 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; Keffaber *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 inappetance 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 viraemic 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 coronaviruses, 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.

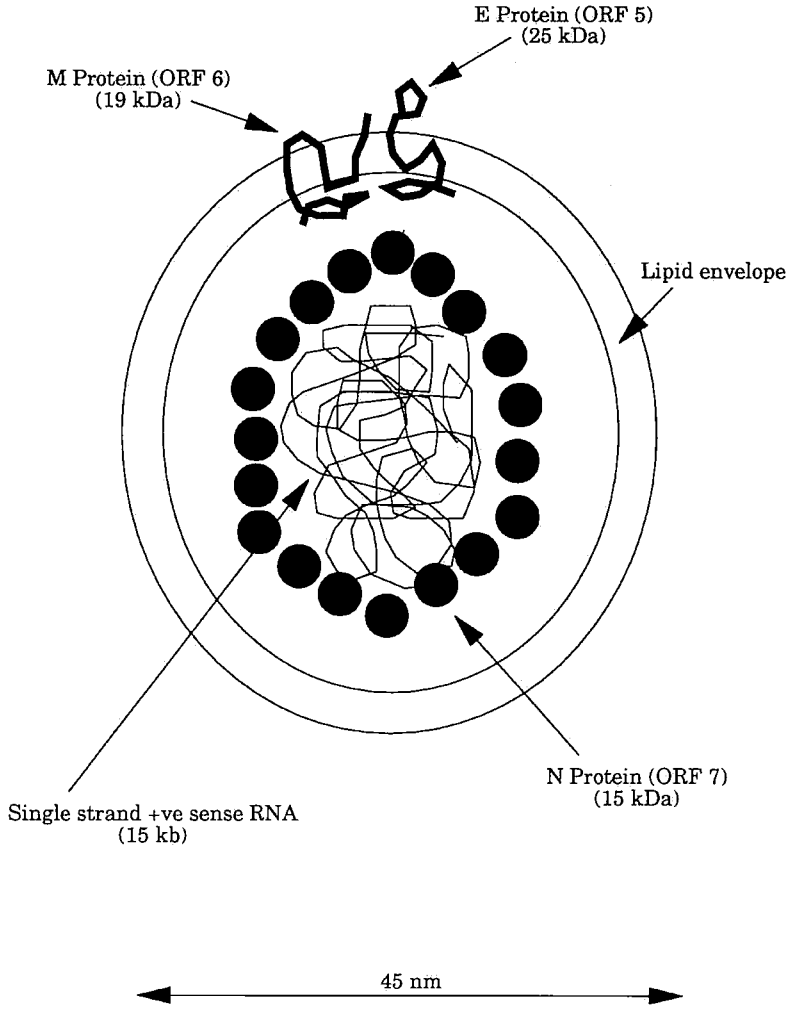

Fig. 2. Schematic diagram of PRRS virion. The product of ORF 3 appears to be virion associated but its topology is uncertain.

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 pneumonic 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 subsequent lactations. Experimental challenge of pregnant sows revealed a 70% protection 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, Gorcyca *et al.* (1995) reported on a live attenuated vaccine (RespPRRS, Boehringer), administered 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 apparently 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 suggested 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 serologically 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 geographical 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" into isolated nurseries can break the cycle of infection from older to younger animals 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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REFERENCES

AHL, R., PENSAERT, M., ROBERTSON, I. B., TERPSTRA, C., VAN DER SANDE, W., WALKER, K. J., WHITE, M. E. & MEREDITH, M. (1992). Porcine reproductive and respiratory syndrome (PRRS or blue-eared pig disease). *Veterinary Record* **130,** 87–9.

ALBINA, E., MADEC, F., CARIOLET, R. & TORRISON, J. (1994). Immune response and persistence of the reproductive and respiratory syndrome virus in infected pigs and farm units. *Veterinary Record* **134,** 567–73.

ALBINA, E., LEFORBAN, Y., BARON, T., PLANA DURAN, J. & VANNIER, P. (1992). An enzyme-linked immunosorbent assay (ELISA) for the detection of antibodies to the porcine reproductive and respiratory syndrome (PRRS) virus. *Annales de Recherches Vétérinaire* **23,** 167–76.

BANE, D., NEUMANN, E., HALL, W., HARLIN, K. & SLIFE, L. (1992). PRRS associated with Fumosin contamination of feed. *American Association of Swine Practitioners Newsletter* **4,** 22-3.

BAUTISTA, E. M., GOYAL, S. M. & COLLINS, J. E. (1993b). Serologic survey for Lelystad and VR-2332 strains of porcine reproductive and respiratory syndrome (PRRS) virus in US swine herds. *Journal of Veterinary Diagnostic Investigation* **5,** 612–4.

BAUTISTA, E. M., GOYAL, S. M., YOON, I. J., JOO, H. S. & COLLINS, J. E. (1993a). Comparison of porcine alveolar macrophages and CL2621 for the detection of porcine reproductive and respiratory syndrome (PRRS) virus and anti-PRRS antibody. *Journal of Veterinary Diagnostic Investigation* **5,** 163–5.

BAUTISTA, E. M., CHOI, C., GOYAL, S. M., MURTAUGH, M. & MOLITOR, T. (1994). Antigenic diversity within porcine epidemic abortion and respiratory syndrome (PEARS) virus isolates. In *Proceedings of the 13th International Pig Veterinary Congress,* Bangkok, Thailand, 26th–30th June, p. 63.

BEILAGE, E., BEILAGE, T., OHLINGER, V., PEARSON J. & BLAHA, T. (1992). Comparative study on the detection of antibodies against the PRRS virus in german breeding and fattening pigs. *American Association of Swine Practitioners Newsletter* **4,** 37.

BENFIELD, D. A., NELSON, E., COLLINS, J. E., HARRIS, L., GOYAL, S. M., ROBISON, D., CHRISTIANSON, W. T., MORRISON, R. B., GORCYCA, D. & CHLADEK, D. (1992). Characterization of swine infer-

tility and respiratory syndrome (SIRS) virus (isolate ATCC VR-2332). *Journal of Veterinary Diagnostic Investigation* **4,** 127–33.

BLACKBURN, P. W. (1991). The impact of the disease on the pig industry—the experience of veterinary practice in the UK. In *Report of the Second Seminar of the EC on Porcine Reproductive and Respiratory Syndrome (the new pig disease),* Brussels, Belgium. PVET/EN/0207, pp. 46–47.

BLAHA, T. (1992). Epidemiological investigations into PEARS in Germany: consequences in fattening pigs. In *Proceedings of the 12th Congress of the International Pig Veterinary Society,* The Hague, Netherlands, 17–20 August, p. 126.

BLOEMRAAD, M., DE KLUIJVER, E. P., PETERSEN, A., BURKHARDT, G. E. & WENSVOORT, G. (1994). Porcine reproductive and respiratory syndrome: temperature and pH stability of Lelystad virus and its survival in tissue specimens from viraemic pigs. *Veterinary Microbiology* **42,** 361–71.

BOTNER, A., NIELSEN, J. & BILLEHANSEN, V. (1994). Isolation of porcine reproductive and respiratory syndrome (PRRS) virus in a Danish swine herd and experimental infection of pregnant gilts with the virus. *Veterinary Microbiology* **40,** 351–60.

BROUWER, J., FRANKENA, K., DE JONG, M. F., VOETS, R., DIJKHUIZEN, A., VERHEIJDEN, J. & KOMIJN, R. E. (1994). PRRS: effect on herd performance after initial infection and risk analysis. *The Veterinary Quarterly* **16,** 95–100.

BRUN, A., VAGANAY, A., TARDY, M. C., NOE, T., VANDEPUTTE, J., SCHIRVEL, C. & LACOSTE, F. (1992). Evaluation of etiological elements in the "P.R.R.S." in pigs. In *Proceedings of the 12th Congress of the International Pig Veterinary Society,* The Hague, Netherlands, 17–20 August, p. 108.

CARLSON, J. (1992). Encephalomyocarditis virus (EMCV) as a cause of reproductive and respiratory disease in swine. *American Association of Swine Practitioners Newsletter* **4,** 23.

CHOI, C., CHRISTIANSON, W., COLLINS, J., JOO, H. & MOLITOR, T. (1992). Antibody-dependent enhancement of SIRS virus replication. *American Association of Swine Practitioners Newsletter* **4,** 30.

CHRISTIANSON, W. T., COLLINS, J. E., BENFIELD, D. A., HARRIS, L., GORCYCA, D. E., CHLADEK, D. W., MORRISON, R. B. & JOO, H. S. (1992). Experimental reproduction of swine infertility and respiratory syndrome in pregnant sows. *American Journal of Veterinary Research* **53,** 485–8.

CHRISTIANSON, W. T., CHOI, C. S., COLLINS, J. E., MOLITOR, T. W., MORRISON, R. B. & JOO, H. S. (1993). Pathogenesis of porcine reproductive and respiratory syndrome virus infection in mid-gestation sows and fetuses. *Canadian Journal of Veterinary Research* **57,** 262–8.

CHRISTIANSON, W. T., CONNOR, J. C., CROWE, C. K., JOO, H. S. & YOON, I. J. (1994). Elimination of PRRS with isowean. In *Proceedings of the 13th International Pig Veterinary Congress,* Bangkok, Thailand, 26–30 June, p. 68.

CLARK, L. K., HILL, M. A., KNIFFEN, T. S., VAN ALSTINE, W., STEVENSON, G., MEYER, K. B., WU, C. C., SCHEIDT, A. B., KNOX, K. & ALBREGTS, S. (1994). An evaluation of the components of medicated early weaning. *Swine Health and Production* **2,** 5–11.

COLLINS, J. E., BENFIELD, D. A., CHRISTIANSON, W., HARRIS, L., GORCYCA, D. E., CHLADEK, D. W. & MORRISON, R. (1991). Swine infertility and respiratory syndrome (mystery swine disease). In *Proceedings of the Minnesota Swine Conference,* University of Minnesota, USA, pp. 200–205.

COLLINS, J. E., BENFIELD, D. A., CHRISTIANSON, W. T., HARRIS, L., HENNINGS, J. C., SHAW, D. P., GOYAL, S. M., MCCULLOUGH, S., MORRISON, R. B., JOO, H. S., GORCYCA, D. & CHLADEK, D. (1992). Isolation of swine infertility and respiratory syndrome virus (isolate ATCC VR-2332) from the disease in gnotobiotic pigs. *Journal of Veterinary Diagnostic Investigation* **4,** 117–26.

COLLINS, J. E. & ROSSOW, K. D. (1993). Pathogenesis of PRRS. In *Proceedings of the Allen D. Leman Swine Conference,* University of Minnesota, USA, pp. 47–48.

DE JONG, M. F., CROMWIJK, W. & VAN'T VELD, P. (1991a). The "new" pig disease; epidemiology and production losses in the Netherlands. In *Report of the First EC Seminar/Workshop on "The New Pig Disease, Porcine Respiration and Reproductive Syndrome",* Brussels, Belgium. PVET/EN/1113, p. 9–19.

DE JONG, M. F., VAN'T VELD, P. & CROMWIJK, W. (1991b). The epidemiology of PRRS in the

Netherlands. In *Report of the Second Seminar of the EC on Porcine Reproductive and Respiratory Syndrome (the new pig disease)*, Brussels, Belgium. PVET/EN/0207-5, p. 2–7.

DEA, S., BILODEAU, R., ATHANASSIOUS, R., SAUVAGEAU, R. & MARTINEAU, G. P. (1992). Swine reproductive and respiratory syndrome in Quebec: isolation of an enveloped virus serologically-related to Lelystad virus. *Canadian Veterinary Journal* **33,** 801–8.

DEE, S. (1992). Investigation of a nationwide outbreak of SIRS using a telephone survey. *American Association of Swine Practitioners Newsletter* **4,** 41–4.

DEE, S. A. & JOO, H. S. (1994a). Prevention of the spread of porcine reproductive and respiratory syndrome virus in endemically infected pig herds by nursery depopulation. *Veterinary Record* **135,** 6–9.

DEE, S. A. & JOO, H. S. (1994b). Recurrent reproductive failure associated with porcine reproductive and respiratory syndrome in a swine herd. *Journal of the American Veterinary Medical Association* **205,** 1017–8.

DIAL, G. D. & PARSONS, T. (1989). SMEDI-like syndrome (EMC?). *Proceedings of the Annual Meeting of the American Association of Swine Practitioners* **20,** 435.

DONE, S. H., PATON, D. J., BROWN, I. & COOLEY, W. (1992). Pathology of porcine reproductive and respiratory syndrome in the UK. In *Proceedings of the 12th Congress of the International Pig Veterinary Society*, The Hague, The Netherlands, 17–20 August, p. 111.

DREW, T. W., MEULENBERG, J. J. M., SANDS, J. J. & PATON, D. J. (1995). Production, characterization and reactivity of monoclonal antibodies to porcine reproductive and respiratory syndrome virus (PRRSV). *Journal of General Virology* (in press).

DYKHUIZEN, A. A., JALVINGH, A. W. & BOLDER, F. W. M. M. (1991). Determining the economic impact of the "new" pig disease. In *Report of the Second Seminar of the EC on Porcine Reproductive and Respiratory Syndrome (the new pig disease)*, Brussels, Belgium. PVET/EN/0207-5, pp. 53–60.

EDWARDS, S., ROBERTSON, I., WILESMITH, J., RYAN, J., KILNER, C., PATON, D., DREW, T., BROWN, I. & SANDS, J. (1992). PRRS ("blue-eared pig disease") in Great Britain. *American Association of Swine Practitioners Newsletter* **4,** 32–6.

FEITSMA, H., GROOTEN, H. J. & SCHIE, F. W. (1992). The effect of porcine epidemic abortion and respiratory syndrome (PEARS) on sperm production. In *Proceedings of the 12th International Congress of Animal Reproduction*, The Hague, The Netherlands 23–27 August, pp. 1710–2.

FICHTNER, D., BERGMANN, S. & SCHIRRMEIER, H. (1994). Einsatz des indirekten immunfluorreszentests zum nachweis von antikorpen gegen das virus des porcine reproductive and respiratory syndrome. *Monatschefte fur Veterinarmedizin* **49,** 223–7.

FIEDLER, J. (1991). Report on the epidemiology of PRRS in Germany. In *Report of the Second Seminar of the EC on Porcine Reproductive and Respiratory Syndrome (the new pig disease)*, Brussels, Belgium. PVET/EN/0207, pp. 13–14.

FRANKENA, K., BROUWER, J., DE JONG, M. F., VOETS, R., VERHEIJDEN, J. & KOMIJN, R. (1992). PRRS: effect on herd performance and risk indicator analysis. In *Proceedings of the 12th Congress of the International Pig Veterinary Society*, The Hague, The Netherlands, 17–20 August, p. 119.

FREESE, W. R., JOO, H. S. & SIMONSON, R. R. (1994). A potential spontaneous elimination of porcine epidemic abortion respiratory syndrome (PEARS) virus spread in a commercial swine herd. In *Proceedings of the 13th International Pig Veterinary Congress*, Bangkok, Thailand, 26–30 June, p. 66.

FREY, M., EERNISSE, K., LANDGRAF, J., PEARSON, J. & CHLADEK, D. (1992). Diagnostic testing for SIRS virus at the national veterinary services laboratories (NVSL). *American Association of Swine Practitioners Newsletter* **4,** 31.

GALINA, L., PILJOAN C., SITJAR, M., CHRISTIANSON, W. T., ROSSOW, K. & COLLINS, J. E. (1994). Interaction between *Streptococcus suis* serotype-2 and porcine reproductive and respiratory syndrome virus in specific pathogen-free piglets. *Veterinary Records* **134,** 60–4.

GODENY, E. K., CHEN, L., KUMAR, S. N., METHVEN, S. L., KOONIN, E. V. & BRINTON, M. A. (1993). Complete genomic sequence and phylogenetic analysis of the lactate dehydrogenase-elevating virus (LDV). *Virology* **194,** 585–96.

GORCYCA, D., SPIESS, D., SCHLESINGER, K., CHLADEK, D., MORRISON, R. & SCHULTZ, R. (1993). The

effect of a previous porcine reproductive and respiratory syndrome (PRRS) virus challenge in pregnant sows after subsequent exposure to virulent virus. In *Proceedings of the American Association of Swine Practitioners*, pp. 51–2.

GORCYCA, D., SCHLESINGER, K., CHLADEK, D. & BEHAN, W. (1995). RespPRRS: a new tool for the prevention and control of PRRS in pigs. In *Proceedings of the American Association of Swine Practitioners*, pp. 1–22.

GORDON, S. C. (1992). Effects of blue-eared pig disease on a breeding and fattening unit. *Veterinary Record* **130,** 513–4.

GOOVAERTS, D. & VISSER, N. (1992). Vascular lesions in pigs infected with PRRS. In *Proceedings of the 12th Congress of the International Pig Veterinary Society.* The Hague, The Netherlands, 17–20 August, p. 129.

GROSSE BEILAGE, E. & GROSSE BEILAGE, T. (1993). Epidemiological investigations in the course of the reproductive performance after PRRS (PEARS) in pig breeding herds. *Deutsche Tierarztliche Wochenschrift* **100,** 32–6.

HALBUR, P. G., PAUL, P. S. & JANKE, B. H. (1993). Viral contributors to the porcine respiratory disease complex. In *Proceedings of the 24th Annual Meeting of the American Association of Swine Practitioners*, Kansas City, Missouri, USA, pp. 343–50.

HALBUR, P., MENG, P. X. & HAGEMOSER, W. (1994a). Marked variability in pathogenicity of nine US porcine reproductive and respiratory syndrome virus (PRRSV) isolates in 5-week-old CDCD pigs. In *Proceedings of the 13th International Pig Veterinary Congress*, Bangkok, Thailand, 26–30 June, p. 59.

HALBUR, P. G., ANDREWS, J. J., HUFFMAN, E. L., PAUL, P. S., MENG, X. J. & NIYO, Y. (1994b). Development of a streptavidin–biotin immunoperoxidase procedure for the detection of porcine reproductive and respiratory syndrome virus antigen in porcine lung. *Journal of Veterinary Diagnostic Investigation* **6,** 254–7.

HARRIS, L., GORCYCA, D., SCHLESINGER, K., SCHULTZ, R., CHLADEK, D., MORRISON, R. & KEFFABER, K. (1992). Case reports of swine infertility and respiratory syndrome (SIRS). In *Proceedings of the 23rd Annual Meeting of the American Association of Swine Practitioners*, Nashville, Tennessee, USA, pp. 96–71.

HOEFLING, D. C. (1990). Mystery swine disease. In *Ninety-fourth annual meeting of the United States Animal Health Association* pp. 501–4.

HOOPER, C. C., VAN ALSTINE, W. G., STEVENSON, G. W. & KANITZ, C. L. (1994). Mice and rats (laboratory and feral) are not a reservoir for PRRS virus. *Journal of Veterinary Diagnostic Investigation* **6,** 13–5.

HOPPER, S. A., WHITE, M. E. C. & TWIDDY, N. (1992). An outbreak of blue-eared pig disease (porcine reproductive and respiratory syndrome) in four pig herds in Great Britain. *Veterinary Record* **131,** 140–4.

HOUBEN, S., CALLEBAUT, P. & PENSAERT, M. B. (1995). Comparative study of a blocking enzyme-linked immunosorbent assay and the immunoperoxidase monolayer assay for the detection of antibodies to the porcine reproductive and respiratory syndrome virus in pigs. *Journal of Virological Methods* **51,** 125–8.

JOO, H. S. & DEE, S. (1993). Recurrent PRRS problems in nursery pigs. In *Proceedings of the Allen D Leman Swine Conference*, University of Minnesota, USA pp. 85–8.

KEFFABER, K. K. (1989). Reproductive failure of unknown aetiology. *American Association of Swine Practitioners Newsletters* **1,** 1–10.

KEFFABER, K., STEVENSON, G., VAN ALSTINE, W., KANITZ, C., HARRIS, L., GORCYCA, D., SCHLESINGER, K., SCHULTZ, R., CHLADEK, D. & MORRISON, R. (1992). SIRS virus infection in nursery/grower pigs. *American Association of Swine Practitioners Newsletter* **4,** 38–40.

KIM, H. S., KWANG, J., YOON, I. J., JOO, H. S. & FREY, M. L. (1993). Enhanced replication of porcine reproductive and respiratory syndrome (PRRS) virus in a homogeneous subpopulation of MA-104 cell line. *Archives of Virology* **133,** 477–83.

KOMIJN, R. E., VAN KLINK, E. G. M. & VAN DER SANDE, W. J. H. (1991). The possible effects of weather conditions on the spread of the "new" pig disease in the Netherlands. In *Report of the First EC seminar/workshop on "The new pig disease, porcine respiration and reproductive syndrome"*, Brussels, Belgium. PVET/EN/1113, pp. 28–31.

KWANG, J., KIM, H. S. & JOO, H. S. (1994). Cloning, expression and sequence analysis of the

ORF4 gene of the porcine reproductive and respiratory syndrome virus MN-1b. *Journal of Veterinary Diagnostic Investigation*, **6**, 293–6.

LAGER, K. M. & ACKERMANN, M. R. (1994). Pulmonary lesions in fetuses exposed in utero to porcine reproductive and respiratory syndrome virus. *Journal of Veterinary Diagnostic Investigation* **6**, 480–3.

LAGER, K. M., MENGELING, W. L. & BROCKMEIER, S. L. (1994). Consequences of porcine epidemic abortion and respiratory virus (PEARSV) infection in bred gilts at different stages of gestation. In *Proceedings of the 13th International Pig Veterinary Congress*, Bangkok, Thailand, 26–30 June, p. 55.

LINDHAUS, W. & LINDHAUS, B. (1991). Tatselhafte sweinekrankheit. *Der Praktische Tierarzt.* **25**, 423–5.

LOULA, T. (1991). Mystery pig disease. *Agri Practice* **12**, 23–34.

MAGAR, R., LAROCHELLE, R., ROBINSON, Y. & DUBEC, C. (1993). Immunohistochemical detection of porcine reproductive and respiratory syndrome virus using colloidal gold. *Canadian Journal of Veterinary Research* **57**, 300–4.

MARDASSI, H., MOUNIR, S. & DEA, S. (1994a). Identification of major differences in the neurocapsid protein genes of a Quebec strain and European strains of porcine reproductive and respiratory syndrome virus. *Journal of General Virology* **75**, 681–5.

MARDASSI, H., WILSON, L., MOUNIR, S. & DEA, S. (1994b). Detection of porcine reproductive and respiratory syndrome virus and efficient differentiation between Canadian and European strains by reverse transcription and PCR amplification. *Journal of Clinical Microbiology* **32**, 2197–203.

MENG, X. J., PAUL, P. S. & HALBUR, P. G. (1994). Molecular cloning and nucleotide sequencing of the 3'-terminal genomic RNA of the porcine reproductive and respiratory syndrome virus. *Journal of General Virology* **75**, 1795–801.

MENGELING, W. L., LAGER, K. M. & VORWALD, A. C. (1994). Temporal characterization of transplacental infection of porcine fetuses with porcine reproductive and respiratory syndrome virus. *American Journal of Veterinary Research* **55**, 1391–8.

MENGELING, W. L., LAGER, K. M. & VORWALD, A. C. (1995). Diagnosis of porcine reproductive and respiratory syndrome. *Journal of Veterinary Diagnostic Investigation* **7**, 3–16.

MEULENBERG, J. J. M., HULST, M. M., DE MEIJER, E. J., MOONEN, P. L. J. M., DEN BESTEN, A., DE KLUYVER, E. P., WENSVOORT, G. & MOORMANN, R. J. M. (1993a). Lelystad virus, the causative agent of porcine epidemic abortion and respiratory syndrome (PEARS), is related to LDV and EAV. *Virology* **192**, 62–72.

MEULENBERG, J. J. M., DE MEIJER, E. J. & MOORMANN, R. J. M. (1993b). Subgenomic RNAs of Lelystad virus contain a conserved leader-body junction sequence. *Journal of General Virology* **74**, 1697–701.

MEULENBERG, J. J. M., PETERSEN-DEN BESTEN, A., DE KLUYVER, E. P., MOORMANN, R. J. M., SCHAAPER, W. M. M. & WENSVOORT, G. (1995). Characterization of proteins encoded by ORFs 2 to 7 of Lelystad virus. *Virology* **206**, 155–63.

MOLITOR, T., LEITNER, G., CHOI, C., RISDAHL, J., ROSSOW, K. & COLLINS, J. (1992). Modulation of host immune responses by SIRS virus. *American Association of Swine Practitioners Newsletter* **4**, 27–28.

MOLITOR, T. (1993). Immune responses to PRRS virus. In *Proceedings of the Allen D Leman Swine Conference*, University of Minnesota, USA, pp. 49–50.

MOORE, C., BILODEAU, R. & WISEMAN, B. (1990). Clinical aspects and consequences of mystery swine disease in nursery and grow-finish. In *MSD Committee Meeting*, Livestock Conservation Institute, Denver, USA, pp. 41–9.

MORRISON, R. B., COLLINS, J. E., HARRIS, L., CHLADEK, D. W., GORCYCA, D. E., JOO, H. S., CHRISTIANSON, W., BENFIELD, D. A., MARSH, W. E., GOYAL, S. & ANNELLI, J. F. (1992a). Sero-epidemiological investigation of porcine reproductive and respiratory syndrome (PEARS, PRRS, SIRS). In *Proceedings of the 12th Congress of the International Pig Veterinary Society*, The Hague, The Netherlands, 17–20 August, p. 114.

MORRISON, R. B., COLLINS, J. E., HARRIS, L., CHRISTIANSON, W. T., BENFIELD, D. A., CHLADEK, D. W., GORCYCA, D. E. & JOO, H. S. (1992b). Serologic evidence incriminating a recently isolated virus (ATCC VR-2332) as the cause of swine infertility and respiratory syndrome (SIRS). *Journal of the Veterinary Diagnostic Investigation* **4**, 186–8.

MORRISON, R., BAUTISTA, E., GOYAL, S., COLLINS, J. & AANNELLI, J. (1992c). Seroprevalence of SIRS virus in the United States in 1990. *American Association of Swine Practitioners Newsletter* **4,** 47.

MORTENSEN, S. & MADSEN, K. S. (1992). PRRS occurrence in Denmark. In *Proceedings of International Symposium on S.I.R.S./P.R.R.S./P.E.A.R.S.*, Minnesota, USA, p. 38.

MURTAUGH, M. (1993). The PRRS virus. In *Proceedings of the Allen D Leman Swine Conference*, University of Minnesota, MN, USA, pp. 43–5.

NELSON, E. A., CHRISTOPHER-JENNINGS, J., DREW, T., WENSVOORT, G., COLLINS, J. E. & BENFIELD, D. A. (1993). Differentiation of United States and European isolates of porcine reproductive and respiratory syndrome virus by monoclonal antibodies. *Journal of Clinical Microbiology* **31,** 3184–9.

OHLINGER, V. F., AHL, R., HAAS, B., METTENLEITER, T. C., RZIHA, H. J., STRAUB, O. C. & WEILAND, F. (1991a). PRRS-Laboratory studies at Tubingen and other German laboratories. In *Report of the Second Seminar of the EC on Porcine Reproductive and Respiratory Syndrome (the new pig disease)*, Brussels, Belgium. PVET/EN/0207, p. 38.

OHLINGER, V. F., WEILAND, F., HAAS, B., VISSER, N., AHL, R., METTENLEITER, T. C., WEILAND, E., RZIHA, H. J., SAALMULLER, A. & STRAUB, O. C. (1991b). Aetiological studies of the porcine reproductive and respiratory syndrome (PRRS). *Tierarztliche Umschau* **46,** 703–4.

OHLINGER, V., WEILAND, F., WEILAND, E., METTENLEITER, T., HAAS B., VISSNER, N. & AHL, R. (1992a). Some aspects of the virus causing PRRS in Germany. *American Association of Swine Practitioners Newsletters* **4,** 16.

OHLINGER, V., HAAS, B., SALLMULLER, A., BEYER, J., TEUFFERT, J., VISSER, N. & WEILAND, F. (1992b). *In vivo* and *in vitro* studies on the immunobiology of pigs. *American Association of Swine Practitioners Newsletter* **4,** 24.

PATON, D. J. & DONE, S. H. (1992). Porcine reproductive and respiratory syndrome ('blue-eared' pig disease). *Veterinary Annual* **32,** 278–92.

PATON, D. J., BROWN, I. H., SCOTT, A. C., DONE, S. H. & EDWARDS, S. (1992a). Isolation of a Lelystad virus-like agent from British pigs and scanning electron microscopy of infected macrophages. *Veterinary Microbiology* **33,** 195–201.

PATON, D. J., DREW, T. W., BROWN, I. H., EDWARDS, S. & DONE, S. (1992b). Laboratory diagnosis of porcine reproductive and respiratory syndrome. *Pig Veterinary Journal* **29,** 188–92.

PATON, D. J. & DREW, T. W. (1995). Serological monitoring of PRRS transmission: a case study. *Veterinary Record* **136,** 297–8.

PIJOAN, C., SOLANO, G. & SEGALES, J. (1994). PRRS virus and secondary disease. In *Proceedings of the Allen D Leman Swine Conference*, University of Minnesota, MN, USA, pp. 225–6.

PLAGEMANN, P. G. W. & MOENNIG, V. (1992). Lactate dehydrogenase-elevating virus, equine arteritis virus and simian haemorrhagic fever virus; a new group of positive-strand RNA viruses. *Advances in Virus Research* **41,** 99–192.

PLANA DURAN, J., VAYREDA, M., VILARRASA, J., BASTONS, M., ROSELL, R., MARTINEZ, M., SAN GABRIEL, A., PUJOLS, J., BADIOLA, J. L., RAMOS, J. A. & DOMINGO, M. (1992). Porcine epidemic abortion an respiratory syndrome (mystery swine disease), isolation in Spain of the causative agent and experimental reproduction of the disease. *Veterinary Microbiology* **33,** 203–211.

PLANA DURAN, J. (1994). Immunoprophylaxis against the porcine reproductive and respiratory syndrome (PRRS) virus. In *PRRS Summit*, North Carolina State University, Raleigh, USA, pp. 1–16.

POL, J. M. A., VAN DIJK, J. E., WENSVOORT, G. & TERPSTRA, C. (1991). Pathological, ultrastructural, and immunohistochemical changes caused by Lelystad virus in experimentally induced infections of mystery swine disease [synonym: porcine epidemic abortion and respiratory syndrome (PEARS)]. *Veterinary Quarterly* **13,** 137–143.

POL, J., WAGENAAR, F., BROEKHUIJSEN-DAVIES, J. & WENSVOORT, G. (1992). The morphogenesis of Lelystad virus in porcine lung alveolar macrophages. In *Proceedings of the 12th Congress of the International Pig Veterinary Society*, The Hague, The Netherlands, 17–20 August, p. 127.

POLSON, D. D., MARSH, W. E., DIAL, G. D. & CHRISTIANSON, W. T. (1992). Financial impact of porcine epidemic abortion and respiratory syndrome (PEARS). In *Proceedings of the 12th Congress of the International Pig Veterinary Society*, The Hague, The Netherlands, 17–20 August, p. 132.

POTTER, R. A. (1994). Non-transmission of porcine reproductive and respiratory syndrome virus by seropositive pigs from an infected herd. *Veterinary Record* **134,** 304–5.

PRIETO, C., SUAREZ, P., SANCHEZ, R., SOLANA, A., SIMARRO, I., MARTIN RILLO, S. & CASTRO, J. R. (1994). Semen changes in boars after experimental infection with porcine epidemic abortion and respiratory syndrome (PEARS) virus. In *Proceedings of the 13th International Pig Veterinary Congress*, Bangkok, Thailand, 26–30 June, p. 98.

RAYMAKERS, R. J. M. L. (1991). The experience of the veterinary practice in Someren (Netherlands) with PRRS. In *Report of the Second Seminar of the EC on Porcine Reproductive and Respiratory Syndrome (the new pig disease)*, Brussels, Belgium. PVET/EN/0207, p. 48.

ROBERTSON, I. B. (1991). The epidemiology of PRRS in the United Kingdom. In *Report of the Second Seminar of the EC on Porcine Reproductive and Respiratory Syndrome (the new pig disease)*, Brussels, Belgium. PVET/EN/0207, pp. 15–21.

ROBERTSON, I. B. (1992a). Porcine reproductive and respiratory syndrome (blue-eared pig disease): some aspects of its epidemiology. In *Proceedings of Society for Veterinary Epidemiology and Preventive Medicine*, Edinburgh, UK, 1–3 April, pp. 24–38.

ROBERTSON, I. B. (1992b). Transmission of blue-eared pig disease (letter). *Veterinary Record* **130,** 478–479.

ROSSOW, K., BAUTISTA, E., GOYAL, S., MURTAUGH, M., MOLITOR, T., MORRISON, R., BENFIELD, D. & COLLINS, J. (1992). The effect of pig age on clinical disease and immunopathogenesis of SIRS virus infection. *American Association of Swine Practitioners Newsletter* **4,** 26.

ROSSOW, K. D., BAUTISTA, E. M., GOYAL, S. M., MOLITOR, T. W., MURTAUGH, M. P., MORRISON, R. B., BENFIELD, D. A. & COLLINS, J. E. (1994). Experimental porcine reproductive and respiratory syndrome virus infection in 1-week-old, 4-week-old, and 10-week-old pigs. *Journal of Veterinary Diagnostic Investigation* **6,** 3–12.

SCHUKKEN, Y. H., DE JONG, M. C. M., KOMIJN, R. E., BRAAMSKAMP, J., DIJKHUIZEN, A. A., NOORDHUIZEN, J. P. T. M., VERNOOY, J. C. M. & VERHEIJDEN, J. H. M. (1992). Porcine epidemic abortion and respiratory syndrome (PEARS)-positive and PEARS-negative diagnoses for breeding and multiplier herds in the Netherlands using statistical methods. *Tijdschrift voor Diergeneeskunde* **117,** 259–64.

SHIMIZU, M., YAMADA, S., MURAKAMI, Y., MOROZUMI, T., KOBAYASHI, H., MITANI, K., ITO, N., KUBO, M., KIMURA, K., KOBAYASHI, M., YAMAMOTO, K., MIURA, Y., YAMAMOTO, T. & WATANABE, K. (1994). Isolation of porcine reproductive and respiratory syndrome (PRRS) virus from Heko-Heko disease of pigs. *Journal of Veterinary Medical Science* **56,** 389–91.

STEVENSON, G. W., VAN ALSTINE, W. G., KANITZ, C. L. & KEFFABER, K. K. (1993). Endemic porcine reproductive and respiratory syndrome virus infection of nursery pigs in two swine herds without current reproductive failure. *Journal of Veterinary Diagnostic Investigation* **5,** 432–4.

STEVENSON, G. W., VAN ALSTINE, W. G. & KANITZ, C. L. (1994). Characterization of infection with endemic porcine reproductive and respiratory syndrome virus in a swine herd. *Journal of the American Veterinary Medical Association* **204,** 1938–42.

STOCKHOFE-ZURWIEDEN, N., CAMARRO, J. A. N., GROSSE-BEILAGE, E., CHAVEZ, J. & POHLENZ, J. (1993). Uterine and placental alterations in pregnant sows associated with the porcine epidemic abortion and respiratory syndrome (PEARS). *Journal of Veterinary Medicine Series B* **40,** 261–71.

SUAREZ, P., ZARDOYA, R., PRIETO, C., SOLANDA, A., TABARES, E., BAUTISTA, J. M. & CASTRO, J. M. (1994). Direct detection of the porcine reproductive and respiratory virus by reverse transcriptase-polymerase chain reaction (RT-PCR). *Archives of Virology* **135,** 89–99.

SWENSON, S. L., HILL, H. T., ZIMMERMAN, J. J., EVANS, L. E., LANDGRAF, J. G., WILLS, R. W., SANDERSON, T. P., MCGINLEY, M. J., BREVIK, A. K., CISZEWSKI, D. K. & FREY, M. L. (1994). Excretion of porcine reproductive and respiratory syndrome virus in semen after experimentally induced infection in boars. *Journal of the American Veterinary Medical Association* **204,** 1943–8.

TERPSTRA, C., WENSVOORT, G. & POL, J. M. A. (1991). Experimental reproduction of porcine epidemic abortion and respiratory syndrome (mystery swine disease) by infection with Lelystad virus: Koch's postulates fulfilled. *Veterinary Quarterly* **13,** 131–6.

TERPSTRA, C., WENSVOORT, G. & LEENGOED, L. A. M. G. (1992). Persistence of Lelystad virus in

herds affected by porcine epidemic abortion and respiratory syndrome. In *Proceedings of the 12th Congress of the International Pig Veterinary Society*, The Hague, The Netherlands, 17–20 August, p. 118.

THACKER, B. (1992). Serological surveys in a herd before, during and after an outbreak of SIRS. *American Association of Swine Practitioners Newsletter* **4,** 40.

TORRISON, J., VANNIER, P., ALBINA, E., MADEC, F. & MORRISON, R. (1994). Incidence and clinical effect of PRRS virus infection in gilts on commercial swine farms. In *Proceedings of the 13th Congress of the International Pig Veterinary Society*, Bangkok, Thailand, 26–30 June, p. 511.

VAN ALSTINE, W. (1991). (MSD) Past diagnostic approaches and findings and potentially useful diagnostic strategies. In *Report of the First EC seminar/workshop on "The new pig disease, porcine respiration and reproductive syndrome"*, Brussels, Belgium. PVET/EN/1113, pp. 71–79.

VAN ALSTINE, W. G., KANITZ, C. L. & STEVENSON, G. W. (1993). Time and temperature survivability of PRRS virus in serum and tissues. *Journal of Veterinary Diagnostic Investigation* **5,** 621–2.

VAN REETH, K., KOYEN, A. & PENSAERT, M. (1994). Clinical effects of dual infections with porcine epidemic abortion and respiratory syndrome virus, porcine respiratory coronavirus and swine influenza virus. In *Proceedings of the 13th International Pig Veterinary Congress*, Bangkok, Thailand, 26–30 June, p. 51.

VAN WOENSEL, P., VAN DER WOUW, J. & VISSER, N. (1994). Detection of porcine reproductive respiratory syndrome virus by the polymerase chain reaction. *Journal of Virological Methods* **47,** 273–8.

VAREWYCK, H. (1991). The epidemiology of PRRS in Belgium. In *Report of the Second Seminar of the EC on Porcine Reproductive and Respiratory Syndrome (the new pig disease)*, Brussels, Belgium. PVET/EN/0207, p. 22.

VOGEL, K., KRAMER, M., LEUFFERT, J. & KRAMER, S. (1991). PRRS-Epidemiological and economic analyses in Germany. In *Report of the Second Seminar of the EC on Porcine Reproductive and Respiratory Syndrome (the new pig disease)*, Brussels, Belgium. PVET/EN/0207, pp. 49–52.

VYNCKIER, C. & PENSAERT, M. (1993). The porcine reproductive and respiratory syndrome: isolation of the virus and epidemiological research in Belgium. *Vlaams Diergeneeskd Tijdschr* **62,** 118–22.

WENSVOORT, G., TERPSTRA, C., POL, J. M. A., TER LAAK, E. A., BLOEMRAAD, M., DE KLUYVER, E. P., KRAGTEN, C., VAN BUITEN, L., DEN BESTEN, A., WAGENAAR, F., BROEKHUIJSEN, J. M., MOONEN, P. L. J. M., ZETSTRA, T., DE BOER, E. A., TIBBEN, H. J., DE JONG, M. F., VAN'T VELD, P., GROENLAND, G. J. R., VAN GENNEP, J. A., VOETS, M. T., VERHEIJDEN, J. H. M. & BRAAMSKAMP, J. (1991). Mystery swine disease in the Netherlands: the isolation of Lelystad virus. *Veterinary Quarterly* **13,** 121–30.

WENSVOORT, G., DE KLUYVER, E. P., LUIJTZE, E. A., DEN BESTEN, A., HARRIS, L., COLLINS, J. E., CHRISTIANSON, W. T. & CHLADEK, D. (1992). Antigenic comparison of Lelystad virus and swine infertility and respiratory syndrome (SIRS) virus. *Journal of Veterinary Diagnostic Investigation* **4,** 134–8.

WHITE, M. E. C. (1992). The clinical signs and symptoms of 'blue-eared pig disease' (PRRS). *Pig Veterinary Journal* **28,** 62–8.

WILLS, R. W., ZIMMERMAN, J. J., YOON, K., SWENSON, S. L., MCGINLEY, M. J., HILL, H. T. & PLATT, K. B. (1995). Porcine reproductive and respiratory syndrome virus: isolation from chronically infected swine. In *Proceedings of the American Association of Swine Practitioners*, pp. 387–9.

YAEGER, M., PRIEVE, T., COLLINS, J., CHRISTOPHER-JENNINGS, J., NELSON, E. & BENFIELD, D. (1993). Evidence for the transmission of porcine reproductive and respiratory syndrome (PRRS) virus in boar semen. *Swine Health and Production* **1,** 7–9.

YOON, J., JOO, H. S., CHRISTIANSON, W. T., KIM, H. S., COLLINS, J. E., CARLSON, J. H. & DEE, S. A. (1992a). Isolation of a cytopathic virus from weak pigs on farms with a history of swine infertility and respiratory syndrome. *Journal of Veterinary Diagnostic Investigation* **4,** 139–43.

YOON, I. J., JOO, H. S., CHRISTIANSON, W. T., KIM, H. S., COLLINS, J. E., MORRISON, R. B. & DIAL,

G. D. (1992b). An indirect fluorescent antibody test for the detection of antibody to swine infertility and respiratory syndrome virus in swine sera. *Journal of Veterinary Diagnostic Investigation* **4,** 144–7.

YOON, I. J., JOO, H. S., CHRISTIANSON, W. T., MORRISON, R. B. & DIAL, G. D. (1993). Persistent and contact infection in nursery pigs experimentally infected with porcine reproductive and respiratory syndrome (PRRS) virus. *Swine Health and Production* **1,** 5–8.

YOON, I. J., JOO, H. S., GOYAL, S. M. & MOLITOR, T. W. (1994). A modified serum neutralization test for the detection of antibody to porcine reproductive and respiratory syndrome virus in swine sera. *Journal of Veterinary Diagnostic Investigation* **6,** 289–92.

ZEMAN, D., NEIGER, R., YAEGER, M., NELSON, E., BENFIELD, D., LESLIESTEEN, P., THOMSON, J., MISKIMINS, D., DALY, R. & MINEHART, M. (1993). Laboratory investigation of PRRS virus infection in three swine herds. *Journal of Veterinary Diagnostic Investigation* **5,** 522–8.

ZHOU, Y., BARGHUSEN, S., CHOI, C., ROSSOW, K., COLLINS, J., LABER, J., MOLITOR, T. & MURTAUGH, M. (1992). Effect of SIRS virus infection on leukocyte populations in the peripheral blood and on cytokine expression in alveolar macrophages of growing pigs. *American Association of Swine Practitioners Newsletter* **4,** 28.

ZIMMERMAN, J. (1991). Mystery swine disease. In *Proceedings of the Annual Meeting of the Livestock Conservation Institute,* Bloomington, MN, USA, 9–11 April.

ZIMMERMAN, J., SANDERSON, T., EERNISSE, K., HILL H. & FREY, M. (1992). Transmission of SIRS virus in convalescent animals to commingled penmates under experimental conditions. *American Association of Swine Practitioners Newsletter* **4,** 25.

ZIMMERMAN, J., SWENSON, S. L., WILLS, R. W., PIRTLE, E. C., YOON, K. J., HILL, H. T. & MCGINLEY, M. J. (1993). Transmission of PRRS virus. In *Proceedings of the Allen D Leman Swine Conference,* University of Minnesota, MN, USA, pp. 51–52.

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