THE PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME
Characteristics and diagnosis of the causative virus

K. Conzelmann(4), H.-J. Thiel(5), E. Albina(6) and T.W. Drew(6)

(1) Central Veterinary Institute, Department of Virology, Houtriweg 39, 8221 RA, Lelystad, The Netherlands
(2) Dept. of Veterinary Pathobiology, Univ. of Minnesota, 1971 Commonwealth Avenue, St. Paul, MN 55108, USA
(3) Dept. of Veterinary Science, South Dakota State Univ., PO Box 2175, N. Campus Drive, Brookings, SD 57007, USA
(4) Federal Research Centre for Virus Diseases of Animals, Paul Ehrlich Strasse 28, D-72076 Tubingen, Germany
(5) Station de Pathologie Porcine, CNEVA, BP 53 F-22440 Ploufragan, France
(6) Central Veterinary Laboratory Weybridge, Woodham Lane, New Haw, Addlestone, Surrey KT15 3NB, United Kingdom

SUMMARY: The newly emerged porcine reproductive and respiratory syndrome (PRRS) is caused by a hitherto unknown virus, the Lelystad virus or PRRS virus. The virus is a small, enveloped, RNA virus which preferentially grows in porcine alveolar macrophages. The virus has a genome of about 15 kb; during its replication a 3' coterminally nested set of six subgenomic RNAs is formed from which the putative structural viral proteins are translated. These molecular characteristics show that the virus can be classified in a new group of viruses, tentatively named the Arterivirus group. European and American strains of the virus are antigenically distinct, and represent two types of the virus. PRRS can be diagnosed by serological as well as virological means and several assays have been developed. However, the current tests should be applied with care, taking into account the antigenic diversity.

RESUME : Le syndrome dysgenesique et respiratoire du porc (SDRP), apparu recemment, est provoque par un virus inconnu ä ce jour, le virus de Lelystad ou virus du SDRP. Il s'agit d'un petit virus enveloppe, à ARN, qui se developpe principalement dans les macrophages alveolaires du porc. Son génome est d'environ 15 kb. Durant la réplication du virus il se forme un ensemble co-terminal 3' de six ARN sous-génomiques, à partir duquel les protéines virales structurales présumées sont synthétisées. Ces caractéristiques moléculaires indiquent que le virus peut être classé dans un nouveau groupe de virus, provisoirement appelé groupe des Arterivirus. Les souches européennes et américaines du virus sont antigeniquement distinctes et représentent deux types de virus. Le SDRP peut être diagnostiqué par des méthodes sérologiques ou virologiques et plusieurs approches ont été développées. Les tests actuels doivent toutefois être utilisés avec précaution, compte tenu de la diversité antigénique.

RESUMEN: El síndrome disgenésico y respiratorio porcino (PRRS), de reciente aparición, es causado por un virus hasta ahora desconocido, el virus Lelystad o virus PRRS. Es un virus pequeño de ARN con envoltura que crece preferentemente en los macrófagos alveolares porcinos. El virus tiene un genoma de aproximadamente 15 kb; durante su replicación se forma un grupo de seis segmentos de ARN ensamblados en forma colineal al extremo 3', a partir de los cuales se traducirían las proteínas estructurales del virus. Estas características moleculares muestran que el virus puede clasificarse en un nuevo grupo de virus, provisionalmente denominado grupo Arterivirus. Las cepas europeas y americana presentan diferencias antigenicas, y constituyen dos tipos de virus. El PRRS puede diagnosticarse mediante varias pruebas serológicas y virológicas, sin embargo habida cuenta de la diversidad antigénica, estas pruebas deben aplicarse con precaución.
INTRODUCTION

The Porcine Reproductive and Respiratory Syndrome (PRRS) which was first described a few years ago is characterized by reproductive failure of sows and respiratory distress of piglets. PRRS has already had a major economic impact on pig production around the world. The disease was first recognized in 1987 in the United States, where it soon was known as Mystery Swine Disease because of the elusive nature of its causal agent, but later was called Swine Infertility and Respiratory Syndrome (SIRS). During the winter of 1990-1991 the disease emerged in Western Europe where it spread rapidly and acquired many more names, including Seuchenhafter Spätabort der Schweine, Abortus blauw, Blue-eared pig disease, Syndrôme Dysgénésique et Respiratoire Porcin, and Porcine Epidemic Abortion and Respiratory Syndrome (PEARS). Throughout this review, we will use the name PRRS for the disease, as this name is most generally accepted by the international veterinary community.

In 1991 the Lelystad virus (LV; Wensvoort et al., 1991a, also known as PRRS virus; Ohlinger et al., 1991, and SIRS virus; Collins et al., 1992) was identified as the causal agent. Subsequently, serological and virological techniques were developed to diagnose the disease. Throughout this review, we will use the name LV as well as PRRSV for the virus, as no general consensus has yet been reached in the scientific community. Furthermore, the taxonomic status of the virus is not yet clear, and in the future it may be appropriate to indicate different antigenic subgroups together with the name of the virus (see below).

The virus grows preferentially in porcine lung macrophages, but only to a limited extent in other cells. Antibodies directed against LV have been detected with a variety of serological tests in sera originating from pigs from the USA, Canada, Germany, the Netherlands, Belgium, Denmark, the United Kingdom, France, Spain, Italy, Poland, Malta, the Philippines and Japan, indicating that LV infections are widespread.

LV (or PRRSV) is a small, positive-stranded and enveloped RNA virus with a genome of about 15 kb, and is tentatively classified as a member of the Arterivirus group (Workshop Arteriviruses, IXth International Congress of Virology, Glasgow, Scotland, 8-13 August 1993). The virus shares many characteristics with other members of this group, such as genome organization, strategy of gene expression, the propensity to grow preferentially in macrophages and a tendency to induce persistent infections. Peculiar for LV is its antigenic diversity, which makes it difficult to diagnose LV infections originating from different countries; especially LV isolates from Europe and the US vary widely (Wensvoort et al., 1992a).

This review reports on several characteristics of the virus, its antigenic diversity and on the serological and virological diagnosis of PRRS.

THE VIRUS

Morphological and biophysical characteristics

LV is a small, enveloped, single-stranded RNA virus that in vitro grows preferentially in porcine alveolar macrophages and in a limited range of other cells (Wensvoort et al., 1991a and 1991b; Ohlinger et al., 1991; Benfield et al., 1992; Nelson et al., 1993). The virus grows with a rapid cytopathic effect in the macrophages. The first signs of cellular degeneration can be observed at three hours after infection. Budding of new virus particles at the smooth endoplasmic reticulum is detected at six hours after infection, and at 12 hours after infection the infected macrophage can already be fully degenerated (Pol et al., 1992). Titres in macrophage cultures reach a maximum of $10^{6.5}$ TCID<sub>50</sub>/ml (Bloemraad et al., 1993). The isolate ATCC VR-2332 can be grown in an established cell line (CL2621) (Collins et al., 1992; Benfield et al., 1992). In these cells it produces a cytopathic effect at 2-4 days after infection and it grows to titres of $10^7$ TCID<sub>50</sub>/ml.
In ultra-thin sections of infected macrophages that are studied by electron microscopy, LV virions appear as 45-55 nm large spherical particles that consist of a 30-35 nm large nucleocapsid surrounded by a rather smooth lipid-bilayer membrane. Negatively stained virions are often ovoid and 50-60 nm in size (Benfield et al., 1992; Wensvoort et al., 1992b). LV is sensitive to treatment with chloroform and has not been shown to haemagglutinate red blood cells. The virus can be stored successfully at -70 and -20°C but loses its infectivity slowly when stored at 4°C (Benfield et al., 1992; Bloemraad et al., 1993). Its buoyant density ranges from 1.14 g/ml on a sucrose gradient to 1.19 g/ml in a caesium-chloride gradient (Wensvoort et al., 1992b; Benfield et al., 1992).

These morphological and biophysical characteristics were strikingly similar to those of members of the Arterivirus group (Plageman and Moennig, 1992), composed of lactate dehydrogenase-elevating virus of mice (LDV), equine arteritis virus (EAV) and simian haemorrhagic fever virus (SHFV), suggesting that LV is related to these viruses which are currently classified within the Togaviridae family.

**Molecular characteristics**

Recently, the nucleotide sequence of the genome of LV has been determined (Meulenberg et al., 1993a). A consecutive sequence of 15,088 bases, followed by a short 3' poly(A) tail, was obtained. Eight open reading frames (ORFs), located on the plus-strand, which might encode virus specific proteins, were identified. ORF1a and ORF1b, located within the 5' terminal 12 kb part of the genome, encode proteins most likely involved in RNA relication and transcription; whereas ORFs 2 to 7, located within the 3' terminal 3.5 kb part of the genome, were predicted to encode putative viral membrane-associated proteins (ORF 2-6) and the nucleocapsid protein (ORF7). Comparisons of the amino acid sequences of ORFs 2-7 (encoding putative structural proteins) with those of another European (Conzelmann et al., 1993) and an American (Murtaugh et al., 1993) isolate of PRRSV revealed a nearly full homology between the European isolates, but a striking heterogeneity between the European and the American isolates, further confirming the antigenic differences which will be discussed below.

Sequence comparisons have indicated that LV is closely related to LDV and EAV but distantly related to coronaviruses and toroviruses (Meulenberg et al., 1993a; Conzelmann et al., 1993; Murtaugh et al., 1993). The order of the genes, i.e. 5'-gene encoding the RNA polymerase - genes encoding the membrane-associated proteins - gene encoding the nucleocapsid protein - 3', is the same in the torovirus Berne virus (BEV; Snijder et al., 1990a and 1990b), EAV (den Boon et al., 1991a), LDV (Plagemann and Moennig, 1992; Godeny et al., 1993; Plagemann et al., 1993), and the coronaviruses (Spaan et al., 1988). The percentage of identical amino acids between the sequences of the viral RNA polymerase, the putative membrane-associated proteins, and the putative nucleocapsid was low between LV and the toro- or coronaviruses but high between LV and the arteriviruses LDV and EAV; it was even higher between LV and LDV (29 to 67%) than between LV and EAV (20 to 36%).

Furthermore, the genomes of LV, EAV, LDV (and the coronaviruses and toroviruses) appear to be similarly expressed. During the replication of LV RNA, a 3' co-terminal nested set of six subgenomic RNAs is formed, which contain an identical leader sequence derived from the noncoding 5' end of the genomic RNA. The specific site, where the leader is fused to the body of the six subgenomic mRNAs, has a conserved sequence of six nucleotides, UCAACC (Meulenberg et al., 1993b). This six nucleotide motif is similar to the junction sequence of LDV and EAV.

The common characteristics of LV, EAV, LDV, and SHFV, namely gene order, strategy of gene expression, and morphological and biophysical characteristics, clearly distinguish this virus group from togaviruses as well as from coronaviruses and warrant the establishment of a new virus family, for which a new name is required (Workshop Arteriviruses, IXth International Congress of Virology, Glasgow, Scotland, 8-13 August 1993).

**Viral proteins**

The putative structural proteins of the virus are translated from the six subgenomic RNAs. For EAV (De Vries et al., 1992), it has been suggested that ORF 2 encodes a minor and ORF 5 a major viral-envelope glycoprotein; that ORF 6 encodes a membrane-spanning protein, and that ORF 7 encodes the nucleocapsid protein of the virus. Considering the amino acid similarities of the various predicted gene
products of the arteriviruses, it seems likely that the corresponding ORFs of LV encode similar products. Furthermore, it was shown by in vitro translation experiments (Meulenberg et al., 1993c) that the ORF 2-5 products are glycosylated, whereas the ORF 6-7 products are not. These data suggest a virus particle containing a nucleocapsid (encoded by ORF 7) surrounded by a viral membrane (containing a membrane-spanning protein, encoded by ORF 6), from which one or more viral-envelope proteins (encoded by ORF 2-5) project.

In cells infected with the Lelystad or the VR-2332 isolates of the virus, at least three viral proteins of 15, 19 and 26 kDa can be detected by radioimmunoprecipitation and Western blotting, using polyclonal pig sera directed against the respective strains (Nelson et al., 1993). The molecular weights of these proteins correspond well with the reported molecular weights of the major structural proteins of the arteriviruses LDV and EAV, which have been reported to contain a nucleocapsid protein of 12-15 kDa, a nonglycosylated membrane-spanning protein of 16-19 kDa and at least one glycosylated envelope protein of 24-44 kDa (Plageman and Moennig, 1992). However, additional studies with gradient purified LV virions and specific antibodies will be necessary to verify that the 15, 19 and 26 kDa proteins found in cell lysates infected with LV are indeed virion structural proteins.

ANTIGENIC DIVERSITY

Recently it was shown that, although the European and US isolates of PRRSV share similar morphological and physico-chemical properties, the various virus isolates from Europe and the United States are antigenically different (Wensvoort et al., 1992a; Nelson et al. 1993; Drew et al., 1993), as determined by serological testing with polyclonal pig sera as well as with mouse monoclonal antibodies. This antigenic diversity was further confirmed by the subsequent comparison of the genomic sequences of various isolates.

Reactivity with pig sera

Initially, when the reactivity of various isolates from the two continents was tested with polyclonal reference sera or field sera, two isolates from the Netherlands and two from Germany resembled each other closely but were antigenically different from three isolates from the United States. Furthermore, the three US isolates differed antigenically among each other (Wensvoort et al., 1992a). In essence, the reference sera that were raised in pigs experimentally infected with either the original European Lelystad virus isolate or with the US ATCC VR-2332 isolate reacted strongly with the homologous but not or weakly with the heterologous virus, and the same was true for the sets of field sera from the two regions. It was concluded that, although the various isolates apparently belong to the same virus species and share a common antigenic denominator, the antigenic differences were too large to rely solely on a serological test employing only one antigenic type of virus to be able to accurately diagnose PRRS.

Monoclonal antibodies

Two research groups have described sets of mouse monoclonal antibodies (MAbs), prepared against US and European isolates of the virus (Nelson et al., 1993; Drew et al., 1993) and directed against the 15 kDa putative nucleocapsid protein, that were able to discriminate between European strains on the one hand and US strains on the other hand. Two MAbs, prepared against the US ATCC VR-2332 isolate, specifically recognized a conserved epitope on the 15 kDa protein, whereas other MAbs, prepared against the South Dakota 1 and Humberside isolates of LV, only recognized epitopes on the US or European strains, respectively.

Genomic diversity

The data obtained with the reference sera and the MAbs against the putative nucleocapsid protein of the virus, point to major differences between isolates of different origin. On top of that, genomic comparison of the European and US strains of the virus indicate that also other putative structural proteins vary widely (Murtaugh et al., 1993). The percentage of predicted amino acid similarities of the ORFs 2-7 between the European Lelystad virus isolate and the US ATCC VR-2332 isolate are
remarkably low (ORF 2, 63%; ORF 3, 60%; ORF 4, 70%; ORF 5, 55%; ORF 6, 79%; ORF 7, 64%), and further confirm that at least two distinct antigenic types of the virus exist. However, although the structural protein sequences are strikingly different, characteristics such as hydrophobicity profiles, predicted glycosylation sites and isoelectric points are largely preserved.

Therefore, the results so far indicate that PRRSV can be divided into two subgroups, with subgroup A representing the European prototype (Lelystad isolate) described by Wensvoort et al. (1991) and subgroup B representing the US prototype (isolate ATCC VR-2332) described by Benfield et al. (1992).

**DIAGNOSIS OF PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME**

**Antibody detection**

Antibodies directed against LV can be detected in various binding assays such as the immunoperoxidase monolayer assay (IPMA) (Wensvoort et al., 1991), the indirect immunofluorescence test (iIFT) (Ohlinger et al., 1991, Yoon et al., 1992), and the enzyme-linked immunosorbent assay (ELISA) (Albina et al., 1992). Furthermore, neutralizing antibodies can be detected in the serum neutralization test (SNT, Morrison et al., 1992) using the SIRS virus isolate and the 2621 cell line. In IPMA and iIFT, antibodies can be detected from days 7 to 14 after infection (Wensvoort et al., 1991; Ohlinger et al., 1991). Titres in the IPMA can be as high 1:10,000 or more. At least 75% of infected sows are still positive in the IPMA at nine months after the initial infection. A comparative trial of ELISA and IPMA gave broad agreement, with both tests demonstrating similar sensitivity and specificity (Albina et al., 1992). However, it was shown for the IPMA, and can be assumed for the ELISA as well, that the early sensitivity can only be counted on provided the test is performed with a test virus that is antigenically similar to the virus inducing the antibodies. If the test virus differs considerably, the antibody response will be detected much later after infection, or be missed altogether (Wensvoort et al., 1992a).

In the SNT, the titres are generally lower than in IPMA and they disappear sooner (Morrison et al., 1992). Due to the nature of the test, it can be expected that the antigenic specificity of the SNT is even narrower than that of the IPMA. Various laboratories can test sera on request (Meredith, 1993); however, the general opinion is that the current available tests can be reliably used for serodiagnosis on a herd basis, but should not be used for individual animal certification.

**Virus detection**

Virus isolation is preferably done on porcine alveolar macrophages or on CL2621 cells. However, the two cell systems are not equally sensitive. Macrophages have, in general, a higher sensitivity to the virus then the CL2621 cell, but some viral isolates grow preferentially in the latter cell (Bautista et al., 1992). Furthermore, the alveolar macrophage cultures used should be tested beforehand for susceptibility to the virus, since not all batches, especially those harvested from pigs older than 6 weeks, support the infection. Not all isolates cause an immediate cytopathic effect, sometimes a second or even third passage is needed. The identity of the newly isolated virus should always be confirmed by immunostaining with specific sera in order to differentiate it from other cytopathic viruses, i.e. pseudorabies virus. During an acute infection (Bloemraad et al., 1993), the virus can be isolated from lung, tonsil, spleen and lymph nodes. However, serum, thoracic and ascitic fluids are the specimens of choice for isolating virus. Virus can be detected in serum for at least three weeks after virus-specific antibodies have been formed (Terpstra et al., 1992).

**DISCUSSION**

This review focuses on recent molecular and virological findings on the Lelystad or PRRS virus. No attention has been given to other points that are of interest with PRRS, e.g. the prevalence and incidence of the disease, the persistence of the virus in the individual pig or in the herd, the duration of the immune response, the question whether or when the immune response is protective, the role of
the macrophage in the pathogenesis of the disease, the mechanisms underlying the reproductive and respiratory aspects of the disease, the possible role of secondary infections, possible differences in virulence of isolates from various areas, and so on. All these aspects await further research, employing well defined tools which are currently being developed. Virus diagnosis is currently hampered by the necessity to use alveolar macrophages, or other susceptible but not widely cell culture systems; now that genomic sequences of the virus have become available it might be worthwhile to investigate whether techniques like polymerase chain reaction (PCR) can provide an answer. The monoclonal antibodies which are currently being developed and characterized will certainly be helpful in further identifying the antigenic differences that exist, and might be used to develop additional diagnostic tools. Such tools will be helpful to study additional aspects of the disease and its causative virus and may help to determine the true significance of PRRS.

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