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Review article

# Lelystad virus and the porcine epidemic abortion and respiratory syndrome

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**Summary** — Lelystad virus is the causal agent of a relatively new pig disease, variously known as mystery swine disease, porcine epidemic abortion and respiratory syndrome and porcine reproductive and respiratory syndrome. The virus is a small, enveloped RNA virus, that tentatively can be included in a new virus family, the Arteriviridae. The virus has infected pigs throughout the world and seems to persist in many pig populations and in individual pigs. Clinical signs of an infection with Lelystad virus are characterized by late term abortions and still birth in sows, and respiratory disease in piglets.

**PEARS / PRRS / arteriviridae / pig**

**Résumé** — **Virus de Lelystad et syndrome dysgénésique et respiratoire du porc.** *Le virus de Lelystad est l'agent causal d'une maladie du porc apparue assez récemment et connue sous le nom de maladie mystérieuse du porc ou syndrome dysgénésique et respiratoire porcine. C'est un petit virus enveloppé à ARN qui peut être inclus provisoirement dans une nouvelle famille de virus, les Artéviridés. Le virus a infecté les porcs dans le monde entier et semble persister à la fois au niveau du troupeau et au niveau de l'individu. Les signes cliniques d'une infection par le virus de Lelystad sont caractérisés par des avortements tardifs et des morts à la naissance chez les truies et des maladies respiratoires chez les porcelets.*

**syndrome dysgénésique et respiratoire porcine / Artéviridé / porc**

## INTRODUCTION

A new pig disease, causing reproductive failures in sows and respiratory problems in piglets, was first noticed in 1987 in the United States (Hill, 1990). In the consecutive years the disease spread through the United States and to Canada. In late 1990 it showed up in Germany after which it spread rapidly through Western Europe.

Initially, the disease was named mystery swine disease because of the elusive nature of its causal agent. A causal role was suspected for many pathogens, among which encephalomyocarditis virus, porcine parvovirus, porcine enteroviruses, swine influenza virus and chlamydia (Joo *et al*, 1990; Mengeling and Lager, 1990; Woolen *et al*, 1990); however, none of these suspicions could be confirmed. As the dis-

ease spread, it acquired more and more names, three of which are most prominent. In the United States it is now known as swine infertility and respiratory syndrome (SIRS) and in Europe it is known as porcine reproductive and respiratory syndrome (PRRS) or as porcine epidemic abortion and respiratory syndrome (PEARS).

Although confusion still reigns in the naming of the disease, several laboratories have isolated its causal agent, the Lelystad virus (LV). This virus was isolated manifold from cases of mystery swine disease in the Netherlands (Wensvoort *et al*, 1991a). The virus was isolated and grew in porcine alveolar macrophages only. In experimentally induced infections, this virus caused reproductive failure in sows (Terpstra *et al*, 1991) and respiratory disease in piglets (Pol *et al*, 1991). Koch's postulates were fulfilled after the virus could be isolated from the experimentally infected animals and LV was thus identified as the causal agent of mystery swine disease. Since the mystery had been solved the disease was renamed PEARs.

LV has also been isolated by laboratories in Germany (Ohlinger *et al*, 1991), Spain (Plana Duran *et al*, 1991), the United Kingdom (Paton *et al*, 1991), France (Albina *et al*, 1992a and 1992b; Baron *et al*, 1992), Denmark (Boetner, personal communication; 1992) and Canada (Dea *et al*, 1992). In the United States, the SIRS virus, which is antigenically and structurally closely related to LV (Wensvoort *et al*, 1992b), was identified as the causal agent of the disease (Collins *et al*, 1992; Christianson *et al*, 1992). Antibodies directed against LV have been detected in sera originating from pigs from all countries listed above and, in addition, in sera from pigs from Belgium, Italy, Poland, Malta and the Philippines, indicating that LV infections are widespread. This review reports on several characteristics of the virus and the disease.

## VIRUS CHARACTERIZATION AND CLASSIFICATION

LV is a small, enveloped, single-stranded RNA virus that *in vitro* grows preferentially in porcine alveolar macrophages and only to low titres in a limited range of other cells (Ohlinger *et al*, 1991; Wensvoort *et al*, 1991a,b). The virus grows with a rapid cytopathic effect in the macrophages. The first signs of cellular degeneration can be observed at 3 h after infection, budding of new virus particles at the smooth endoplasmic reticulum is detected at 6 h after infection, and at 12 h 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. The SIRS virus isolate ATCC VR-2332 of LV can be grown in an established cell line (CL2621) (Benfield *et al*, 1992; Collins *et al*, 1992). In these cells it shows a cytopathic effect at 2–4 d after infection and it grows to titres of  $10^7$  TCID<sub>50</sub>/ml. Recently it was shown, that although LV and the SIRS virus are the same virus, the various isolates from Europe and the United States can differ antigenically. Two isolates from the Netherlands and 2 from Germany resembled each other closely but were antigenically different from 3 isolates from the United States. The 3 US isolates differed antigenically among each other (Wensvoort *et al*, 1992).

LV is sensitive to treatment with chloroform and has not been shown to hemagglutinate red blood cells. The virus can be stored well at  $-70$  and  $-20$  °C and loses its infectivity slowly when stored at 4 °C (Benfield *et al*, 1992; Wensvoort, unpublished results). 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*, 1991b; Benfield *et al*, 1992). In none of the available tests to detect antibodies directed against LV (see below)

does the virus react with various anti-sera directed against a wide range of known animal viruses (Ohlinger *et al*, 1991; Wensvoort *et al*, 1991b; Albina *et al*, 1992c; Benfield *et al*, 1992).

In ultra-thin sections of LV-infected macrophages that are studied by electronmicroscopy, LV virions appear as 45–55 nm large spherical particles that consist of a 30–35 nm large nucleocapsid which is surrounded by a lipid bilayer membrane. Negatively stained LV particles are often ovoid and 50–60 nm large (Benfield *et al*, 1992; Pol, unpublished observations). LV has tentatively been classified in the family Togaviridae (Benfield *et al*, 1992), and it has been suggested that LV, together with equine arteritis virus, lactic dehydrogenase virus and simian hemorrhagic fever virus can be grouped in the genus *Arterivirus* (Ohlinger *et al*, 1991; Wensvoort *et al*, 1991b).

Recently, the nucleotide sequence of the genome of LV has been determined (Meulenber *et al*, 1992). A consecutive sequence of 15 088 bases was obtained. Eight open reading frames (ORFs) that might encode virus-specific proteins were identified. ORF1a and ORF1b likely encode the viral RNA polymerase, whereas ORFs 2 to 6 were predicted to encode viral membrane-associated proteins. The nucleocapsid protein is most likely encoded by ORF7. The order of the genes, *ie* 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, b), equine arteritis virus (EAV; den Boon *et al*, 1991a), lactate dehydrogenase-elevating virus (LDV; Plagemann and Moennig, 1992), and the coronaviruses (Spaan *et al*, 1988). Furthermore, the genomes of LV, EAV, LDV and the coronaviruses appear to be similarly expressed. During the replication of LV RNA, a 3' co-terminal nested

set of 6 subgenomic RNAs is formed, which contain an identical leader sequence derived from the noncoding 5' end of the genomic RNA. Sequence comparisons have indicated that LV is closely related to LDV and EAV and distantly related to coronaviruses and toroviruses (Meulenber *et al*, 1992). The percentage of identical amino acids between the sequences of the viral RNA polymerase, the putative membrane-associated proteins, and the putative nucleocapsid was even higher between LV and LDV (29–67%) than between LV and EAV (20–36%). Recently, it has been suggested that LV, together with equine arteritis virus, lactate dehydrogenase-elevating virus and simian hemorrhagic fever virus can be grouped in the genus *Arterivirus* (Meulenber *et al*, 1992; Plagemann and Moennig, 1992). These 4 viruses share enough common characteristics, and are sufficiently different from the other genera in the Togaviridae, to warrant a tentative inclusion in a new virus family, Arteriviridae.

## EPIDEMIOLOGY

Lelystad virus infections and antibodies directed against LV have been detected in pig herds in the USA, Canada, the UK, the Netherlands, Germany, Denmark, Poland, Belgium, France, Spain, Italy and the Philippines, therefore, in short, one can find PEARS in many of the major pig producing areas throughout the world.

A first outbreak of PEARS can spread rapidly and with great economic damage through an uninfected area; in the Netherlands the infection spread in 6 weeks from the east to the west of the country and 400 000 piglets (of a total yearly production of 20 million) were reportedly lost during that period (Wensvoort, EEG). In Ger-

many, the disease spread in a similarly vigorous way (Ohlinger *et al*, 1991) whereas in other countries less densely populated with pigs, such as the UK (Paton *et al*, 1991) and France (Albina *et al*, 1992a), it has spread less rapidly. Transmission of the virus by air and transmission through pig-to-pig contacts have to be considered as the major routes of infection (Komijn *et al*, 1991). Spread of the virus *via* human-to-pig contacts, *via* infected meat or meat-products or *via* sperm has not been reported. Spread within a herd is fairly rapid. Terpstra *et al* (1992) reported that up to 85–95% of sows seroconverted to LV within 2–3 months after the introduction of the virus in a breeding herd. However, once a pig herd has become infected the infection seems to persist in the herd. Several mechanisms can be held responsible for the persistence of the virus in the breeding herd (Terpstra *et al*, 1992). Firstly, during a primary outbreak of PEARS up to 15% of the sows may escape initial infection. Secondly, LV-infected litters can be born up to 10 wk after an initial outbreak, and thirdly, individual pigs can transmit LV to susceptible contacts for up to 8 wk postinfection. Since part of the herd will escape initial infection and a pig herd has a continuous input of susceptible stock and because of the presence of short-term carriers, the virus can persist in the herds. In one herd which was monitored in the above study, a virus positive litter was born 5 months after the initial outbreak from a sow which had remained seronegative for at least 4 months. If LV infections persist in herds then one can conclude that once an area is infected the infection most likely will become enzootic in those areas. In the same study, it was reported that 1 year after the initial outbreak, 3–5-month-old replacement gilts were found seropositive for LV on all 6 farms tested, indicating that the virus had indeed persisted in those 6 herds.

## CLINICAL SIGNS

The clinical signs of PEARS are most prominent in breeding herds that have not yet been infected before with LV. In such herds the disease is characterized by mass reproductive failure that lasts from 1–3 months (Collins *et al*, 1991; Ohlinger *et al*, 1991; Wensvoort *et al*, 1991a).

The onset of the infection in sows is often marked by anorexia, which, if it occurs on a large scale in the herd, can be very specific. Some sows demonstrate respiratory distress, and some have slightly elevated body temperatures. Blue discoloration of ears, abdomens and vulvas has been reported from Europe only and seems to occur in 1% of the cases (De Jong, 1991). Pregnant sows will abort late (d 107–112) in gestation, or have high numbers of stillborn, mummified or weakly-born piglets that die shortly after birth. In experimental infections (Ohlinger *et al*, 1991; Terpstra *et al*, 1991; Christianson *et al*, 1992) with LV, the above clinical signs have been fully reproduced. In all 3 experiments pregnant sows were infected in the last third part of gestation varying from d 84–93. In general, the onset of anorexia and fever was seen within 2–4 d after intranasal inoculation, and respiratory distress and blue discolorations were seen between d 6–12. Late abortions were reported from gestation d 107 and on, but the majority of the sows farrowed on time and gave birth to stillborn and weak piglets from which the virus could be isolated.

Piglets that are infected in the farrowing house shortly after birth will often develop signs of respiratory disease and sometimes die (Collins *et al*, 1991; Ohlinger *et al*, 1991; Wensvoort *et al*, 1991a). The affected piglets become anorexic, have a transient fever, show respiratory distress, have roughened hair coats and grow slow-

ly. In these piglets a distinct interstitial pneumonia can be seen (Collins *et al*, 1991). Often, the disease symptoms seem to be worsened by secondary infections of a varied nature. Experimentally infected gnotobiotic piglets (Pol *et al*, 1991; Collins *et al*, 1992) developed the pulmonic lesions within 2 d after infection but did not show pronounced respiratory distress, again indicating that secondary infections with other pathogens may play an important role.

Older piglets and fattening pigs seem less affected by LV infections, often a short period of anorexia and mild respiratory distress are the only signs that are reported. Whether the infection can be seriously aggravated by secondary infections and thus result in poor growth and deaths among fattening pigs remains still an open question.

## **PATHOGENESIS**

Little is yet known about the pathogenesis of the disease. Since the porcine alveolar macrophage is preferentially infected by LV this cell most likely stands central in the infection. In *in vitro* studies (Pol *et al*, 1992), LV was shown to kill alveolar macrophages within 12 h after infection, and in *in vitro* immunohistochemical studies of experimentally infected gnotobiotic piglets, Pol *et al* (1991) showed that the alveolar macrophages contain LV antigen and become degenerated. Other prominent findings, however, were degeneration of epithelial cells of the nasal mucosa and the bronchii, an interstitial pneumonia characterized by cellular infiltration in the alveolar septa, and lymphoid depletion of spleen, thymus, tonsil and lymphnodes. In a similar study, Collins *et al* (1992) found similar lesions and evidence of encephalitis.

## **IMMUNE RESPONSES**

Antibodies directed against LV can be detected in various binding assays such as the immunoperoxidase monolayer assay (IPMA) (Wensvoort *et al*, 1991a), the indirect immunofluorescence test (iIFT) (Ohlinger *et al*, 1991; Yoon *et al*, 1992), and the enzyme-linked immunosorbent assay (ELISA) (Albina *et al*, 1992b, c). Furthermore, neutralizing antibodies can be detected in the neutralization test (SNT, Morrison *et al*, 1992) using the SIRS virus isolate and the 2621 cell line. In IPMA and iIFT, antibodies directed against LV can be detected from d 7–14 on after infection (Ohlinger *et al*, 1991; Wensvoort *et al*, 1991a). However, it was shown for the IPMA that this early sensitivity can only be counted on provided the test is performed with a test virus that is antigenically the same as the virus to which the animals have produced the antibodies. If the test virus is different, then the antibody response will be detected much latter after infection, or will be missed altogether (Wensvoort *et al*, 1992). Titres in the IPMA are high, they can reach up to 1:10 000 or even higher (Wensvoort *et al*, 1991a). At least 75% of infected sows is still positive in IPMA at nine months after the initial infection. In the SNT, the titres are generally lower than in IPMA and they disappear sooner (Morrison *et al*, 1992), and it can be expected that the antigenic specificity of the SNT is even narrower than that of the IPMA. Nothing is yet known about the antigenic specificity of the ELISA.

## **CONCLUSION**

Now that the causal agent of the mysterious swine disease has been identified, research can foremost be aimed at characterizing the virus. A complete charac-

terization of its genome will enable us to clarify whether the LV is indeed a new member of the Artiriviridae, of which the other members are the lactate dehydrogenase-elevating virus, the equine arteritis virus and the simian hemorrhagic fever virus. These viruses, which replicate *via* a characteristicly nested set of messenger RNA's (Plagemann and Moennig, 1992), have several peculiar properties in common. They seem to be very species-specific and even replicate preferentially in one cell-type, the macrophages. Furthermore, they can vary in virulence and are sometimes able to persistently infect their host. A better understanding of LV and the other 3 viruses will help us better understand PEARS, a disease which seems to be affecting more and more countries in the world.

Major questions are whether indeed the virus can persist in the pig herd, and whether the disease and damage that result from such enzootic infections will occur at a large scale. Early findings in the Netherlands seem to indicate that although the virus remains in the pig herds, the damage resulting from PEARS has greatly been reduced. Furthermore, we need to investigate whether PEARS remains restricted to breeding herds—where an enzootic infection with LV could give similar damage as an infection with the porcine parvovirus—or whether also fattening herds are hampered by the disease.

Characterizing the virus will also enable us to develop better diagnostic tools to diagnose PEARS. Although the existing serologic techniques seem to be performing well once an LV infection has established itself in a certain region, the antigenic variability of the virus will make it difficult to always accurately diagnose a primary outbreak. We need to identify a common antigen of LV to which pigs infected with various antigenic variants will always respond with the formation of antibodies.

Furthermore, characterization of the various antigens and proteins of LV will also be helpful in developing a vaccine against PEARS.

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