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Report on the first outbreaks of the porcine reproductive and respiratory syndrome (PRRS) in France. Diagnosis and viral isolation

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Summary — We describe the first known occurrence in France of the porcine reproductive and respiratory syndrome (PRRS), a new porcine disease that first appeared in Germany in November 1990. Outbreaks of the disease appeared in November 1991 in Brittany (France), with comparable clinical features to those observed in other countries, were definitively confirmed by a serological analysis from the affected animals using an immunoperoxidase monolayer assay with PRRS-virus infected alveolar macrophages. Furthermore, we report the isolation from one serum of a filtrable agent that, in view of its cytopathic effect for porcine alveolar macrophages and of the serologic reactivity of the infected cells, appears similar to the Lelystad virus that has been implicated in the etiology of the disease.

porcine reproductive and respiratory syndrome / mystery swine disease / blue ear disease / swine / Immunoperoxidase

Résumé — Description des premiers foyers de syndrome dysgénésique et respiratoire porcin (SDRP) en France. Diagnostic et isolement viral. Nous rapportons la première démonstration de l'apparition en France du syndrome dysgénésique et respiratoire du porc (SDRP), une nouvelle maladie porcine initialement apparue en Allemagne en novembre 1990. Les cas de la maladie, qui présentent des caractéristiques cliniques comparables à celles observées dans les autres pays, ont été confirmés par l'étude des sérums des animaux malades par la technique d'immunoperoxydase sur des macrophages alvéolaires de porcs, infectés par le virus SDRP. Par ailleurs, nous décrivons l'isolement, à partir d'un sérum, d'un agent filtrable qui apparaît similaire, par ses effets cytopathiques sur les macrophages alvéolaires de porc et par la réactivité sérologique des cellules infectées, au virus de Lelystad, impliqué dans l'étiologie de la maladie.

syndrome dysgénésique et respiratoire porcin / maladie mystérieuse du porc / maladie des oreilles bleues / porc / Immunoperoxydase

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INTRODUCTION

A new porcine disease, the porcine reproductive and respiratory syndrome (PRRS), suddenly appeared in Germany in November 1990 (Lindhaus and Lindhaus, 1991). The disease then spread to the Netherlands (Cromwijk, 1991), Belgium, Great Britain (Anonymous, 1991), and Spain (San Gabriel, 1991). Since 1987, a quite similar disease of unknown etiology and thus referred to as "Mysterious pig disease", has also affected the United States (Zimmerman, 1991) and Canada (Martineau *et al*, 1991).

The disease, that causes a respiratory syndrome followed by reproductive disorders (abortion, premature birth, high mortality of piglets), has spread widely throughout the United States, Canada, Germany and the Netherlands.

The isolation of a viral etiologic agent, unrelated to the other known porcine viruses and designated as Lelystad virus, was first reported by Wensvoort in the Netherlands (Wensvoort *et al*, 1991) then was also isolated in Spain (Plana Duran *et al*, 1991). This agent, which may belong to the Arterivirus group (Ohlinger *et al*, 1991), allowed experimental reproduction of the disease (Terpstra *et al*, 1991; Pol *et al*, 1991; Plana Duran *et al*, 1991).

In this report, we present the first clinical and serological evidence of the occurrence of the porcine reproductive and respiratory syndrome in France, as well as the isolation of one strain of an agent antigenically related to the Lelystad virus.

MATERIALS AND METHODS

Sample collection

Pig sera were collected from 4-breeding-finishing farms (1 to 4) in which clinical signs

similar to those described in porcine reproductive and respiratory syndrome (PRRS) (anorexia, fever and respiratory disorders; abortion, premature birth and high piglet mortality) were detected in November 1991. The number of tested sera and the types of farms from which the animals were sampled are summarized in table 1.

Besides sera from sows sampled after the disease in these 4 breeding-finishing farms, other sera were also available from animals sampled 2 months before the onset of the disease in farm 1, and from animals that entered inside farm 2 only 1 day before sampling.

Pig sera were also collected from 2 fattening units (farms 5 and 6) in the neighbourhood of farms 1 and 2 respectively, in which the pigs only showed respiratory disorders.

Histological examination of lungs showing macroscopic pneumonia lesions, was performed by Dr G Plassiard (École Vétérinaire, Nantes, France) on 4 piglets from farm 1.

Virus and cell cultures

Porcine alveolar macrophages collected from specific pathogen-free (SPF) pigs available in the laboratory were seeded in 96 micro-well titre plates with 10^5 cells/well in 100 μ l of Eagle's Minimal Essential Medium supplemented with heat-inactivated fetal calf serum (10%), penicillin (200 U/ml), streptomycin (0.2 mg/ml) and polymyxine B (80 U/ml). These cells were infected with culture supernatants from porcine alveolar macrophages inoculated with a PRRS virus strain isolated in Spain (Plana Duran *et al*, 1991). This virus strain (Ref 218) was isolated from the lungs of a 1-day-old piglet from one of the Spanish outbreaks. It was shown to be antigenically related to the Lelystad virus by G Wensvoort from Central Veterinary Institut Lelystad, The Netherlands (personal communication). It was provided to us at its fourth passage on porcine alveolar macrophages. Infectious culture supernatants had been prepared by freezing infected macrophage cultures when a > 70% cytopathic effect was reached; these supernatants were repeatedly frozen and thawed, and used at a dilution of 1/50 for the inoculation of fresh macrophage cultures in the micro-well titre plates. After overnight incubation, the infected

cells were fixed with cold paraformaldehyde (4%) in phosphate buffered saline (PBS) for 5 min and the plates were kept frozen at -20°C until use.

Serological examination

The sera were tested by an immunoperoxidase monolayer assay as previously described (Wensvoort *et al*, 1991). Briefly, formaldehyde fixed previously infected alveolar macrophages were incubated with 2-fold serial dilutions of sera (routinely 1/100 to 1/800) for 1 h at 37°C . All the dilutions of sera were performed in PBS supplemented with Tween 20 (0.1%) and horse serum (4%). After washing, monolayers were incubated with horseradish peroxidase-conjugated rabbit antiserum (Dako, Trappes, France) to swine immunoglobulins (1/200) for 1 h at 37°C ; appropriate dilution of conjugated antiserum was chosen by preliminary examination of serial dilutions. After washing, staining was revealed by 3-amino-9-ethyl carbazole (Ref A5754, Sigma, La Verpillière, France). Negative control sera were SPF-pig serum available in the laboratory and hyperimmune sera prepared and tested against other known porcine viruses (Aujeszky's disease, hog cholera, Talfan's disease, transmissible gastroenteritis, epidemic diarrhoea, influenza H1N1 and H3N2, respiratory coronavirus, parvovirus, rotavirus, adenovirus, encephalomyocarditis virus, hemagglutinating encephalitis virus). The positive control serum against PRRS-virus, provided by Dr Plana Duran, was from a sow (Ref 76) experimentally infected with the virus; it was shown to be negative against pseudorabies and porcine influenza viruses and to react with PRRS-virus infected porcine alveolar macrophages, at 1/800 dilution in our experiments.

Viral isolation

Attempts to isolate a viral strain were performed with serum and plasma samples from farm 4. Eight fresh sera and 4 fresh plasma samples were inoculated in 96 micro-well titre plates at 2-fold step dilutions (1/2 to 1/128) on fresh cultures of alveolar macrophages from SPF-pigs,

seeded as previously described. Cultures were regularly observed for the appearance of cytopathic effects. They were frozen when such an effect was observed and had reached 50–70% of the cells. Serial passages were then performed with repeatedly frozen and thawed, diluted (1/50) supernatants from these cultures, on fresh alveolar macrophages cultures. At passage No 3, the viral titre ($\text{TCID}_{50}/\text{ml}$) of the supernatants was estimated by the method of Kaerber (Kaerber, 1931). Furthermore, the serologic examination of infected cells was also performed at passage 3 as previously described.

RESULTS

Clinical and serological observations

Clinical signs observed in farms 1 to 3 were initial influenza syndrome in sows (anorexia, fever and respiratory disorders with coughing and sneezing) followed by reproductive disorders (abortion, premature birth, weakness and high piglet mortality). Transient blue discolouration of the ears was also observed. In the farm 4, the sera were sampled at the time of the onset of the disease, which appeared with reproductive disorders only. In the 2 fattening units (5,6), the clinical signs observed were respiratory disorders and some transient blue discolourations of the ears. Pneumonia lesions were histologically confirmed as due to interstitial pneumonia from all the piglet lungs examined.

Serological examination of the sera sampled after the disease showed as the positive control serum, an intense red cytoplasmic staining of some cells in the immunoperoxidase monolayer assay. As summarized in table 1, such staining was observed with all the examined sera from sows in farms 1 to 3. In farms 5 and 6 respectively, 9 out of 17 and 16 out of 25 sera from breeding pigs were positive. In a

Table 1. Serologic results for porcine reproductive and respiratory syndrome in 6 French farms.

<i>Farm</i>	<i>Type of farm</i>	<i>No of sera tested</i>	<i>No of positive sera</i> ^a
1	Breeding-finishing farm	12 5 ^b	12 0
2	Breeding-finishing farm	8 3 ^c	8 0
3	Breeding-finishing farm	5	5
4	Breeding-finishing farm	10	0
5	Fattening farm	17	9
6	Fattening farm	25	16

^a Positive sera show specific staining at sera dilutions $\geq 1/200$; ^b sera from sows sampled 2 months before the onset of the disease; ^c sera from gilts inside the farm for 1 day before sampling.

number of sera, a positive staining was still observed at a dilution of 1/800, as with the positive control serum. The control cells, which were uninfected by the virus, did not show such staining. Furthermore, in farm 1, the 5 tested sera which had been sampled 2 months before the onset of the disease and in farm 2, the sera from the 3 examined animals which entered the farm only one day before sampling remained negative. Furthermore, the 10 sera examined from the animals on farm 4 which had been sampled at the beginning of the outbreak were also found to be negative in this assay. The negative control serum from SPF pigs and all examined hyperimmune sera against other known porcine viruses were negative. Serologic results of field cases were confirmed by the study of 10 sera (5 positive and 4 negative field sera in our study from farms 1 or 2, as well as control negative SPF serum) tested by G Wensvoort (personal communication).

Viral isolation

Cultures of porcine alveolar macrophages with 8 sera and 4 plasma from affected sows, sampled in farm 4 at the onset of the disease, showed a cytopathic effect with one of the sera (1264), identical to that observed with the reference strain from Spain. Serial inoculation of fresh cultures with the obtained supernatants reproducibly led to a similar cytopathic effect, clearly demonstrable within 48 h, which completely destroyed the monolayer within 5 days. Such a cytopathic effect was retained after filtration at 0.22 μ m of the supernatants. The viral titre was $10^{5.7}$ TCID₅₀/ml at passage 3. The serological examination of the infected cells with the immunoperoxidase monolayer assay showed a similar reactivity to that observed with the virus strain from Spain, as determined with 8 positive and 2 negative field sera as well as with the negative SPF or positive control sera.

Moreover, these field sera included 5 positive and 2 negative sera that were previously confirmed in the laboratory by G Wensvoort, with the PRRS-virus reference strain isolated in the Netherlands (personal communication).

DISCUSSION

We describe the first known outbreak of the porcine reproductive and respiratory syndrome (PRRS) in France, determined by serological confirmation. Furthermore, we report the isolation of an infectious agent similar to the Lelystad virus. This virus, first described as the etiologic agent of the disease in the Netherlands (Wensvoort *et al*, 1991), was recognized as implicated in the disease in other European countries, while the disease affecting North America since 1987 was also considered to be similar. The identification of the PRRS-virus as a new virus was supported by the absence of sero-conversion against previously known porcine viruses (data not shown). The absence of cross-reactivity of the 2 studied strains from Spain and France with any of the examined specific sera against other known porcine viruses in our experiments should be emphasized. Finally, the present observations in France, and particularly the fact that a seroconversion could be established in one of the affected farms after the disease, further argues for the causative role of this virus in field cases as in other European countries.

The clinical features of the disease have previously been described as differing somewhat within countries and farms; however, they are always essentially characterized by reproductive disorders (abortion, premature birth, weakness and high piglet mortality) preceded by an in-

fluenza syndrome (anorexia, fever, and respiratory disorders) (de Jong *et al*, 1991; Van Alstine, 1991). Transient blue discolourations of the ears, abdomen or vulva, that led to the designation of the disease as "abortus blauw" or "blue ear disease", have also been regularly described in some animals. These main clinical features of the disease are the same as those we observed.

Studies are currently in progress to follow the possible progression of the disease in France, which could be associated with aerial diffusion of the virus (Komijn *et al*, 1991) that led to the spread of the disease over a wide geographical area in some other countries.

Attempts to isolate a viral strain from French field cases allowed the identification of an agent similar to the previously described Lelystad virus (Wensvoort *et al*, 1991). It was obtained from one of the sera sampled at a farm at the onset of clinical signs and before the appearance of any serologic evidence of the disease by the immunoperoxidase monolayer assay. Interestingly, it must be emphasized that seroconversion at this farm was confirmed 3 weeks later by an ELISA test that has been developed for the diagnosis of the disease in our laboratory (Albina *et al*, 1992). This filtrable agent, present in the supernatants from serum-inoculated porcine alveolar macrophages cultures, and appears antigenically related to the PRRS-virus reference strains described in the Netherlands (Wensvoort *et al*, 1991) and in Spain (Plana Duran *et al*, 1991).

The identification of PRRS-cases in France is of importance because of the potential economic impact of the disease in pig production, and the isolation of a viral strain similar to those obtained in other countries provides a new tool for further study of the disease.

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REFERENCES

- 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. *Ann Rech Vét* 23, 167-176
- Anonymous (1991) Fears of "blue ear" disease in Humberside. *Vet Rec* 128, 511
- Cromwijk W (1991) The new pig disease – further observations in Dutch herds. EEC Sem Rep New Pig Disease: Porcine Reproductive and Respiratory Syndrome (PRRS), Brussels, 29–30 April 1991, 20-27
- De Jong MF, Cromwijk W, Van't Weld P (1991) The new pig disease: epidemiology and losses in the Netherlands. EEC Sem Rep New Pig Disease: Porcine Reproductive and Respiratory Syndrome (PRRS), Brussels, 29-30 April, 9-19
- Kaerber G (1931) Beitrag zur kollektiven Behandlung pharmakologischer Reinhenversuche. *Arch Exp Pathol Pharmacol* 162, 480-483
- Komijn RE, Van Klink EGM, Van der Sande WJH (1991) The possible effects of weather conditions on the spread of the new pig disease in the Netherlands. EEC Sem Rep New Pig Disease: Porcine Reproductive and Respiratory Syndrome (PRRS), Brussels, 29-30 April, 28-31
- Lindhaus W, Lindhaus B (1991) Rätselhafte Schweinekrankheit. *Prakt Tierarzt* 5, 423-425
- Martineau GP, Bilodeau R, Sauvageau R, Moore C, Dea S (1991) Syndrome "Hyperthermie–Anorexie–Avortement de la truie" et de pneumonie interstitielle virale au Québec. *Journ Rech Porcine Fr* 23, 179-188
- Ohlinger VF, Ahl R, Haas B, Mettenleiter TC, Rziha HJ, Straub OC, Weiland F (1991) PRRS—laboratory studies at Tübingen and other German laboratories. EEC Sem Rep New Pig Disease: Porcine Reproductive and Respiratory Syndrome (PRRS), Brussels, 4-5 November
- Plana Duran J, Vayreda M, Vilarrasa J, Bastons M, San Gabriel A, Rosell R, Martinez M, Pujols J, Domingo M (1991) Isolation and identification in Spain of the agent responsible for the porcine reproductive and respiratory syndrome (PRRS). Abstr 2nd Congr Eur Soc Vet Virol, Uppsala (Sweden), 23–26 September
- Pol JMA, Van Dijk JE, Wensvoort G, Terpstra C (1991) Pathological, ultrastructural, and immunohistochemical changes caused by Lelystad virus in experimentally induced infections of mystery swine disease. *Vet Q* 13, 137-143
- San Gabriel A (1991) Abortion in sows: a laboratory problem. EEC Sem Rep New Pig Disease: Porcine Reproductive and Respiratory Syndrome (PRRS), Brussels, 29–30 April, 62-64
- Terpstra C, Wensvoort G, Pol JMA (1991) Experimental reproduction of porcine epidemic abortion and respiratory syndrome (mystery swine disease) by infection with Lelystad virus: Koch's postulates fulfilled. *Vet Q* 13, 131-136
- Van Alstine WG (1991) Mystery pig disease in the United States. EEC Sem Rep New Pig Disease: Porcine Reproductive and Respiratory Syndrome (PRRS), Brussels, 29-30 April, 65-70
- Wensvoort G, Terpstra C, Pol JMA, Ter Laak EA (1991) Mystery swine disease in the Netherlands: the isolation of Lelystad virus. *Vet Q* 13, 121-130
- Zimmerman J (1991) Mysterious swine disease. Proc Anim Meet 9–11 April Livestock Conversion Institute Bloomington, MN, USA, 8