Persistence of Porcine Reproductive and Respiratory Syndrome Virus Infection in a Swine Operation

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ABSTRACT

A herd of Quebec seedstock pigs experienced in early 1992 a typical outbreak of porcine reproductive and respiratory syndrome (PRRS) associated with lesions of interstitial, proliferative and necrotizing pneumonia in weaned piglets. The nature of the infection was confirmed by serology using indirect immunofluorescence (IIF) and virus isolation in primary cultures of porcine alveolar macrophages (PAM). Farm production recovered after eight weeks of losses. In order to evaluate the persistence of infection in the herd, five SPF-piglets were introduced in two different sections of the PRRS-affected barn four months after the disappearance of clinical symptoms, and two others were placed in a neighboring building with apparently healthy farrow-to-finish pigs. Clinical signs, body temperature, humoral immune response, virological and histopathological findings were recorded over a 42-day period. Clinical signs were evident in all of the sentinels and prolonged fever (≥40°C) was recorded one day post-exposure (PE). Antibody titers to PRRS virus could be detected by IIF on PAM seven days PE, and reached 1:1024 by day 21 PE. Three of the sentinels developed significant virus neutralizing antibody titers (>1:8 to ≤1:128) by day 35 PE. In all cases, the virus could be isolated from the serum between day 7 and 42 PE. Thus, the virus and specific antibodies coexisted for several weeks. Lesions of interstitial pneumonia was demonstrated in few animals. In experimental inoculation studies, the viral strain isolated from the sentinel pigs produced severe reproductive disorders in two sows inoculated at 95 days of gestation. This study confirms that subclinical PRRS virus infection persists for a significant period in different age groups and locations and may, therefore, be transmitted by contact to replacement animals or via seedstock sold to healthy farms.

RÉSUMÉ

Les adultes d'un élevage sélectionneur du Québec furent aux prises au début de 1992 avec une infection typique du syndrome reproducteur et respiratoire porcin (SRRP) associé à des lésions de pneumonie interstitielle, prolifératives et nécrosante. Le sérotypage par immunofluorescence indirecte (IIF) et l'isolement du virus en cultures primaires de macrophages alvéolaires de porc permirent de confirmer le diagnostic. Après huit semaines de pertes, les symptômes disparurent. Afin d'évaluer si le virus persistait toujours dans l'élevage, cinq porcelets furent introduits dans deux différentes sections du bâtiment affecté par le SRRP, quatre mois après la disparition des symptômes cliniques. D'autre part, deux porcelets témoins furent introduits dans un bâtiment voisin abritant un élevage apparemment sain de type naisseur-finisseur. Les symptômes cliniques, la température corporelle, la réponse immunitaire humorale, les suivis virologiques et pathologiques furent effectués sur une période de 42 jours. Déjà, après une journée d'exposition, des porcelets montrèrent des signes cliniques d'infection dont une fièvre prolongée (≥40°C). Après sept jours d'exposition, des titres en anticorps anti-virus SRRP étaient décelables par IIF chez tous les animaux sentinelles; après 21 jours, les titres pouvaient atteindre 1:1024. Trois des animaux sentinelles avaient des titres en anticorps neutralisants significatifs contre le virus (>1:8 à ≤1:128) après 35 jours. A partir du jour 10 et jusqu'à la fin de la période d'observation (J42), il fut possible d'isoler le virus du sérum des animaux sentinelles. Ainsi, le virus persista dans la circulation malgré la présence simultanée de hauts titres d'anticorps pendant plusieurs semaines. Lors des examens histopathologiques, des lésions de pneumonie interstitielle ont été démontrées chez quelques animaux. Il a été possible de reproduire des désordres reproducteurs chez des truies gestantes de 95 jours inoculées par la voie intranasale à l'aide de l'isolat du virus SRRP cultivé à partir d'homogénats des poumons et sérums des animaux sentinelles. Ces travaux confirment qu'en dépit d'absence de signes cliniques pour une période assez longue, le virus SRRP peut persister dans l'élevage chez les animaux de différentes sections de la ferme, et être transmis potentiellement par contact aux animaux de remplacement vendus aux fermes saines.

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Funded in part by the Ministère de l'Agriculture, des Pêcheries et de l'Alimentation du Québec (MAPAQ), la Fédération des Producteurs de Porcs du Québec, Vetrepharm Research Inc., London, Ontario, and the Conseil des Recherches en Pêcherie et en Agro-alimentaire du Québec.

Submitted October 13, 1993.

INTRODUCTION

In the winter 1991–1992, there was still confusion about the case definition of mystery swine disease (MSD) in Canada despite the distinction between two new pathological entities: porcine reproductive and respiratory syndrome (PRRS) and proliferative and necrotizing pneumonia (PNP). The general description of these two new diseases overlapped and caused confusion for veterinarians and breeders as to which etiological agent was responsible for different losses (1–3). Absence of reliable diagnostic tools left only clinical descriptions and pathological lesions as aids to identify the infectious agents and determine control strategies.

Since the initial report on the isolation of a new swine *Arterivirus* related to PRRS in Europe (Lelystad virus) (4–6), confirmation of its pathogenic potential was made in the U.S. (7–9) and the province of Québec (10). The development of indirect immunoperoxydase monolayer assay (IPMA), indirect immunofluorescence (IIF), and enzyme-linked immunosorbert assay (ELISA) tests for the detection of anti-PRRSV antibodies made possible the identification of both acutely- or chronically-infected herds (6,11,12,30). On the other hand, a new antigenic variant of swine influenza virus (SIV) type A was believed to be the causative agent of PNP in Québec (13,14). However, the concomitant infection of many Québec swine herds with both PRRSV and the new antigenic variant of SIV (1,2), plus the occurrence of interstitial pneumonia (IP) and PNP in the same herds, complicated considerably the differential diagnosis (2,15). For example, secondary bacterial infections were frequent in PRRS, but also in PNP, and were attributed to the impairment of the immune system of pigs of various ages (16–18). Since serological investigations showed no evidence of reduction in the humoral immune response of naturally- or experimentally-infected pigs (11,12, 18,19), cell mediated immunity was logically considered as being potentially affected (20,21).

The objective of this field study was: 1) to describe the agents involved in a typical outbreak of PRRS associated with IP-PNP; 2) to conduct a cohort study on sentinel pigs introduced in the farm several weeks after the end of noticeable clinical signs and production losses; 3) to follow the humoral immune response of the exposed piglets and finally 4) to confirm experimentally the pathogenicity of the virus for healthy pregnant sows and piglets.

TABLE I. Variations in the clinical parameters during an eight-week observation period following onset of PRRS outbreak

Symptoms	Farm A	Farm B	Clinical criteria used in Quebec to define PRRS in 1991 (1)
Fever/anorexia in adults	>20 %	<2 %	Moderate to high
Abortion rate	10 %	<1 %	>5 %
Stillbirth rate	31 %	5 %	>15 %
Preweaning mortality	35 %	11 %	>20 %
Pneumonia	PPN/PI	nil	positive

PNP = Proliferative and necrotizing pneumonia
PI = Interstitial pneumonia

TABLE II. Comparison of productivity parameters before, during, and after PRRS in farms A and B

Productivity parameters		Period (month/year) 10/90 to 09/91 (Before)	10/91 to 03/92 (During)	04/92 to 03/93 (After PRRS Outbreak)
No. of litters	(A)	716	320	763
	(B)	633	321	641
Births/litter	(A)	10.4	10.2	9.9
	(B)	9.8	9.8	9.6
% stillbirths	(A)	9.3	16	8.3
	(B)	5.1	5.9	7.1
% preweaning	(A)	15.3	23.5	13.3
mortality	(B)	11.2	11	11.5
No. weaned/litter	(A)	8.2	5.9	7.6
	(B)	8.5	8.4	8.3
Weaned/sow/year	(A)	19.1	16.7	17.5
	(B)	20.6	20.9	20.6

MATERIALS AND METHODS

SELECTION OF THE HERDS

The PRRS-affected herd was a conventional farrow-to-finish purebred operation of 285 sows health-certified by a government monitoring program since its repopulation from several conventional healthy sources in new all-in all-out facilities in 1987. The herd was serologically free of pleuropneumonia associated to *Actinobacillus pleuropneumoniae* type 1 and 5, transmissible gastroenteritis virus (TGEV), and clinically free of swine dysentery (*Serpulina hyodysenteriae*), salmonellosis, swine influenza and atrophic rhinitis. Monitoring of the herd production since its origin by a computerized production analysis program (PATPQ) revealed no history of significant reduction in farrowing, reproductive or weaning data except a steady rate of stillbirth losses (8–9%) considered above the targeted 5% level.

During a period of eight weeks between late December 1991 and early February 1992, a sudden outbreak of fever/anorexia in adults, abortion and premature farrowing, stillborn or autolyzed fetuses and preweaning mortality swept through the barn and progressively subsided after a diagnosis of reproductive impairment, associated with parvovirus (PPV) and *Streptococcus suis*, was given by the diagnostic laboratory. Diagnosis of PPV infection was based on direct fluorescent antibody (FA) on frozen tissue sections from stillborn piglets (22). The syndrome also included dyspnea in suckling piglets associated with lesions of diffuse interstitial pneumonia. Nevertheless, the clinical definition of PRRS in Quebec at that time made this case appear suspicious (Table I). Later, a feverish piglet was submitted for necropsy and histopathological diag-

nosis of PNP was made without virological confirmation of the presence of swine influenza virus (13). Table II summarizes the productivity during a 30 month period in barn A where the syndrome appeared and barn B, a neighboring farrow-to-finish F-1 building situated 50 meters from barn A; movement of animals was from A to B, regularly.

SELECTION OF SENTINEL PIGS

Seven pigs of SPF origin were selected from a farm free of atrophic rhinitis, *Mycoplasma hyopneumoniae*, TGEV, SIV, encephalomyocarditis virus (EMCV), *S. hyodysenteriae*, sarcoptic mange, salmonellosis, *A. pleuropneumoniae* and PRRSV. Three animals were four weeks of age and two were seven weeks of age. All the sentinels were clinically healthy before they were moved to the case farm and separated in one nursery room and one grower-finish section, respectively. Two similar piglets were introduced in the clinically-healthy neighboring barn B. The SPF pigs were injected with long acting penicillin on day 0 and 2, fed a commercial diet medicated with therapeutic levels of tiamulin (75 ppm) and oxytetracyline (330 ppm), and were housed in a pen with no direct contact with neighboring pigs. The rectal temperature was monitored daily for 21 days postexposure, and other clinical signs were followed for three more weeks.

LABORATORY INVESTIGATIONS

The monitoring period of sentinel pigs lasted 42 days during which the animals were bled weekly for serological investigations. The presence of antibodies to TGEV and EMCV was determined by virus neutralization (VN) (23), and antibodies to the Québec antigenic variant of swine influenza A virus IAF-5393 were detected by hemagglutination inhibition (HAI) tests (13). A blocking enzyme-linked immunosorbent assay (ELISA) was used for the detection of antibodies against porcine respiratory coronavirus (PRCV) (24). Indirect immunofluorescence (IIF) tests for the detection of anti-PRRSV antibodies were conducted as described, using PAM cells infected with the reference Québec isolate IAF-exp91 of PRRSV (10). A clone of MA-104 cell line (MARC-145), supporting replication of PRRSV, was kindly provided by Dr. J. Kwang (U.S. Meat Animal Research Center, Clay Center, Nebraska) and used for determination of neutralizing antibodies to PRRSV (25). The antibody titers in the VN and HAI tests were expressed as the reciprocal of the highest serum dilution that inhibited 400 $TCID_{50}$ or four HA units of virus. Negative and positive reference sera were included in each test.

Necropsy was performed on a feverish and dyspneic sentinel pig (#11) ten days after its introduction in the farm, while the six remaining pigs were necropsied on day 42. Tissues were fixed in 10% buffered formalin, and paraffin sections were stained with hematoxylin and eosin. Lung, heart, bronchial lymph node and liver tissues were examined for the presence of microscopic lesions. Frozen sections of these tissues were submitted to IIF staining for viral antigens; rabbit hyperimmune sera to *Mycoplasma hyopneumoniae*, SIV, TGE, PPV and PRRSV were used as primary antiserum in the IIF tests.

To attempt virus isolation, ten percent tissue homogenates were prepared in Eagle's minimal essential medium with Earle's salts, glutamine, and antibiotics, and clarified by centrifugation at 5,000 × *g* for 20 min. Aliquots (0.5 mL) of clarified supernatant fluids, as well as heat-inactivated serum collected weekly from sentinel pigs, were inoculated into the allantoic cavity of 11-day-old embryonated hens' eggs and onto confluent monolayers. Continuous monkey kidney (Vero), dog kidney (MDCK), porcine fallopian tube (PFT) and porcine testicular cells (PT) were used. The cell cultures were propagated as described (26,27). Primary cultures of porcine alveolar macrophages (PAM) were prepared according to the procedure described by Wensvoort *et al* (6). After an absorption period of one hour at room temperature, the infected cell monolayers were rinsed twice with PBS and reincubated at 37°C in culture medium without fetal bovine serum. Cultures were monitored daily for the appearance of cytopathic effects (CPE). Subpassages were done at five to six day intervals, depending on the extent of CPE. For virus identification, the cells were fixed at 24–36 h PI, with 80% acetone in PBS, and stained by IIF using anti-IAF exp91 rabbit hyperimmune serum, or monoclonal antibody SDOW17 (kindly provided by Dr. Benfield of South Dakota State University) directed against the nucleocapsid protein of ATCC VR-2332 strain of PRRSV, and fluorescein-conjugated antirabbit or antimouse IgG (10).

EXPERIMENTAL INOCULATION OF GESTATING SOWS

Three SPF sows, originating from a PRRS seronegative herd, were introduced at 85 days of gestation in isolated experimental quarters. One sow was kept as a control animal and two others were inoculated intranasally at 95 days of gestation with clarified supernatant fluid (5 mL per nostril; infectivity titers 10^5 $TCID_{50}$/mL) of PAM cells that had been inoculated with serum from exposed sentinels pigs. Complete destruction of the monolayers was usually seen within 72 to 96 hours postinfection. The sows were monitored for body temperature, appetite and clinical signs as well as outcome of farrowing. Blood samples were collected at entry, at farrowing day (PID 21) and at postmortem (PID 42). Tissues of the fetuses, live born or nursing piglets aged seven days or less and sows were analyzed for histopathological and virological purposes.

RESULTS

CONFIRMATION OF A DIAGNOSIS OF PRRSV INFECTION

Consulting veterinarians, as well as those involved in the government health monitoring program, feared that PRRS or PNP was responsible for the reproductive and respiratory problems that had occurred in the farm. Before approving sale of seedstock to clients, they needed confirmation as to which etiological agent was involved and if convalescent pigs represented a potential source of contamination for naive animals.

Preliminary analysis of tissues and sera from aborted fetuses and weak piglets allowed the isolation of a cytopathic strain of PRRSV on PAM;

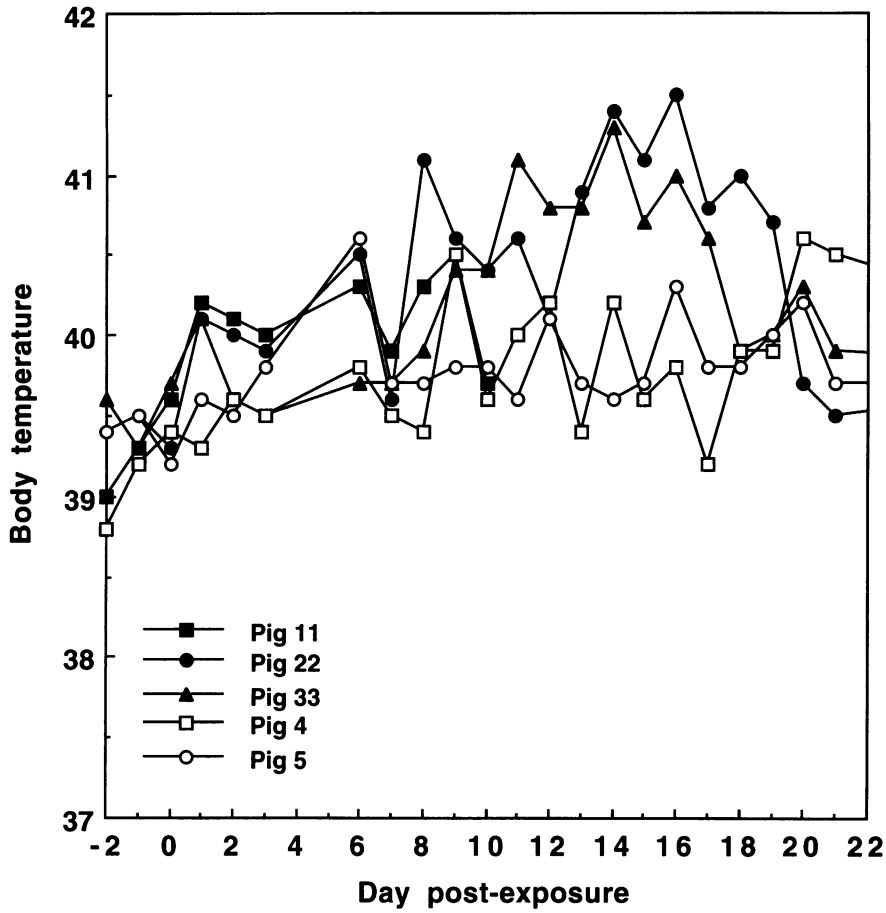

Fig. 1. Changes in body temperature over time in five sentinel SPF pigs exposed to the PRRSV-infected herd. The animals developed fever 24 h after their introduction into the herd. Body temperature rose over 40°C in all pigs, but pigs # 11, 22 and 33 introduced into the nursery section were affected for a longer time.

the virus was identified by IIF tests using anti-PRRSV MAb SDOW17. Attempts to isolate other porcine viruses in Vero cells and continuous pig cell cultures were unsuccessful. Fluorescent antibody examination of fetal tissues and lungs of sick unweaned piglets for EMCV, SIV and TGEV was negative, whereas PPV could be detected in frozen tissue sections from a stillborn pig that had been submitted for necropsy. Serological analysis of paired sera from aborted sows and dyspneic piglets showed seroconversion to PRRSV (IIF titers >1:16), but neither significant antibody titers nor seroconversion were demonstrated to SIV and other porcine viruses tested.

RETROSPECTIVE AND PROSPECTIVE SEROLOGY

Sera collected nine months (fall 1991) before the onset of clinical disease and nine months after disappearance of clinical signs (fall 1992) were analyzed to assess the status of barn A (PRRS-affected) and barn B (clinically healthy). During this 18 month period, neither barn had growing-finishing pigs with acute respiratory signs. Furthermore, the onset of clinical signs in breeding females was not accompanied by a change in the serological status to the viruses, including PRRSV, of animals in the finishing sections. Serological investigations conducted at the onset of clinical signs showed that 100% of sows tested (10/10) from barn A, and 62.5% of those tested (5/8) from barn B, were seronegative to PRRSV. No significant antibody titers to SIV, TGEV and PPV were demonstrated before and after the PRRS outbreak in sows of both barns, but most were seropositive to PRCV by the blocking ELISA test (data not shown). Retrospective serological investigations revealed that nine months before the onset of reproductive problems in sows, growing pigs were already seropositive to PRRSV with IIF antibody titers varying from 1:16 to 1:256 (according to Dr. W.R. Freeze, Oxford Laboratories, Wortington, Minnesota). Nine months following the episode of reproductive problems, antibody titers to PRRSV in the growing section were still high (antibody titers >64).

CLINICAL SIGNS IN SENTINEL PIGS INTRODUCED IN THE PRRS-AFFECTED BARN

The five sentinel pigs, introduced in two different sections of the PRRS-affected barn A, had normal appetite and body condition. However, body temperature exceeded 40°C in all pigs, and the pigs in the nursery section were affected for a longer period. As illustrated in Fig. 1, sentinel piglets developed fever 24 h after their introduction, which persisted from day 9 to day 21 PE. Respiratory signs were noticed in all sentinel pigs; labored breathing lasted for a short period and seemed most severe in nursery pigs from day 7 to day 14 PE, whereas in growers, it was most evident from day 7 to day 10 PE. No other significant clinical sign was noticed.

The two sentinels placed in the neighboring barn B (clinically healthy) experienced the same infection with similar results, as well as *Mycoplasma hyopneumoniae* and *Actinobacillus pleuropneumoniae* type 3 chronic infections.

MACROSCOPIC AND HISTOLOGICAL LESIONS IN SENTINAL PIGS IN THE PRRS-AFFECTED BARN

On day 10 PE, one nursery piglet (#11) was submitted for necropsy with signs of dyspnea and fever that lasted at least three days. Neither macroscopic nor histological lesions were noticed with the exception of a mild chronic rhinitis.

On day 42 PE, the four remaining sentinel pigs introduced in barn A were euthanized. Gross lesions were confined to the respiratory tract. The lungs were tan and partly collapsed with occasional small anteroventral areas of congestion and consolidation. The mediastinal lymph nodes were enlarged and edematous. There was also a moderate atrophy of the ventral nasal turbinates. Microscopically, all

sentinel pigs displayed a light to moderate multifocal interstitial pneumonia characterized by mononuclear cells within the alveolar septa, occasional serocellular exudate in the alveolar lumen, but no hyperplasia of type II pneumocytes (Fig. 2). The presence of a moderate peribronchiolar and perivascular lymphoplasmocytic cell infiltration was also noted, as well as, hyperplasia of the lymphoid follicles in the spleen.

VIROLOGICAL AND SEROLOGICAL INVESTIGATIONS

A cytopathic virus was isolated in primary cultures of PAM from sera collected from all of the sentinel piglets between day 7 and day 42 PE as well as from lung homogenates of piglets that were euthanized at the end of the observation period. Identification of PRRSV was confirmed by IIF using MoAb directed against the nucleocapsid protein of the reference ATCC VR2332 strain. An intense cytoplasmic fluorescence was also observed following incubation of infected PAM with hyperimmune rabbit serum directed to the reference Québec IAF-exp91 isolate (10). No other virus could be isolated from the clinical specimens in other cell lines or embryonated hen's eggs. No virus was seen in clarified supernatant fluids or clarified allantoic fluids by negative stain electron microscopy or hemagglutination-inhibition.

Serologically, none of the five sentinel pigs introduced in barn A developed significant antibodies directed against EMCV, PPV, SIV, and TGEV during the 42 day observation period. On the other hand, IIF antibody titers to PRRSV were first detected at day 7 PE in two nursery pigs and one growing pig. In both cases, the highest antibody titers (1:512 to 1:2048) were determined between day 21 and day 28 PE (Table III). Similarly, the two sentinel pigs, in barn B got the same infection with similar serological results. Comparatively, only three of the sentinel pigs in barn A developed significant anti-PRRSV neutralizing antibodies (SN titers > 1:8 to ≤ 1:128); these antibodies could not be detected before day 35 PE.

EXPERIMENTAL INOCULATION STUDY

In order to determine if the PRRSV strain isolated from sentinel pigs was virulent, pregnant sows were housed in isolated experimental quarters and ten days later, they were inoculated intranasally at 95 days of gestation with clarified supernatant fluids of infected PAM cells. The first inoculated sow showed a transient fever on days 2, 4, 14, 17 and 25. Swelling and reddening of the vulva, and marked congestion of nipples could be observed between days 12 and 20 PI (data not shown). The sow farrowed on day 115 and delivered three liveborn, two stillborn and seven autolyzed piglets. The second infected sow had fever on day 3 (39.5°C) and showed no skin discoloration. On day 115, she farrowed two liveborn, one stillborn, one mummy and seven autolyzed piglets. PRRSV was isolated from lung homogenates on both sows euthanized on day 28 and 42 PI, respectively. The virus could also be isolated in cultures of PAM from tissues of stillborn and weakborn fetuses, but not from the mummified or autolyzed fetuses. Significant antibody titers to PRRSV were detected in sera collected from infected sows at day of farrowing, as well as in the case of live piglets, but not in stillborn fetuses.

Comparatively, the control sow remained clinically normal during the observation period, did not farrow prematurely, and delivered ten live pigs on day 115 of gestation. The animal displayed no loss of appetite or fever, despite a chronic joint illness,

TABLE III. Antibody titers to PRRSV in sentinel pigs place in a herd with PRRS

Sentinel animals	Barn	Days postexposure						
		0	7	14	21	28	35	42
2	"B"	<1:8	1:8	1:256	1:256	1:256	1:256	1:512
3		<1:8	<1:8	1:256	1:256	1:512	1:256	1:512
4	"A"	<1:8	1:8	1:128	1:512	1:1024	1:512	1:1024
5		<1:8	1:16	1:128	1:512	1:512	1:1024	1:512
11		<1:8	1:32	—	—	—	—	—
22		<1:8	1:32	1:512	1:2048	1:1024	1:512	1:1024
33		<1:8	1:8	1:128	1:2048	1:512	1:1024	1:512

Antibody titers to PRRS virus were determined by IIF
Negative or nonsignificant (<1:16) Ab titers to EMCV or TGEV by serum neutralization
Negative or nonsignificant (<1:20) Ab titers to SIV by HAI
Declining maternal Ab (< 1:80) titers to PPV were demonstrated by HAI

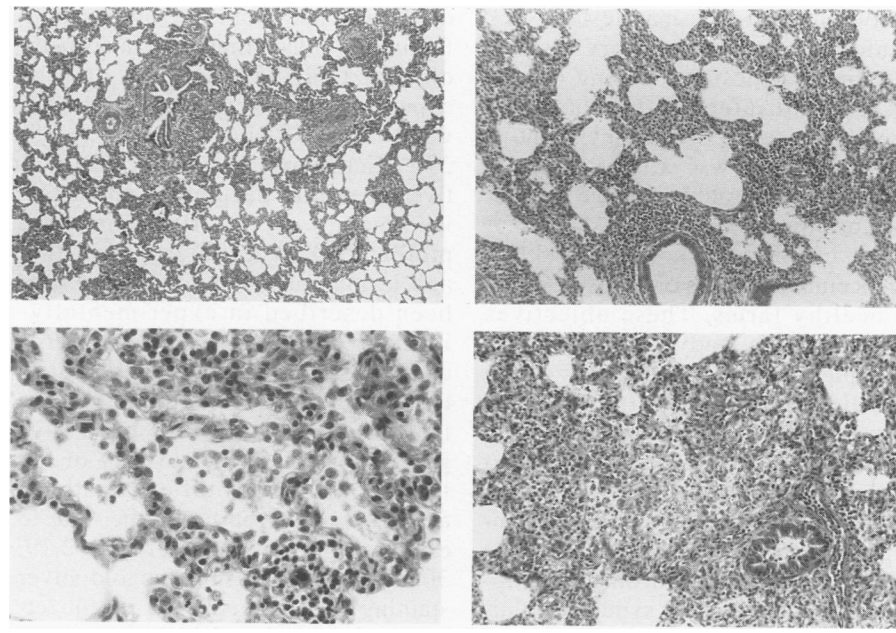

Fig. 2. Histopathological findings in sentinel pigs 42 days after their introduction in the PRRS-positive farm. A,B. General aspect of interstitial pneumonitis with alveolar septa thickened by mononuclear cell infiltration, peribronchiolar lymphocytic cell infiltration and vascular cuffing. C. Free macrophages in alveolar lumen but no pycnotic cells and no evidence of alveolar epithelization. D. Proteinaceous exudate and accumulation of mononuclear cells and necrotic cell debris within the alveolar lumen. H & E stain.

and showed no seroconversion to PPV, TGE, EMC, SIV or PRRSV.

DISCUSSION

The objectives of the present study were to identify the infectious agents responsible for dramatic reproductive losses in a Québec pig farm characterized by severe systemic signs in adults and absence of lesions in the affected sows, aborted fetuses and stillborn pigs. Following or during this period of reproductive failure, postweaning piglets manifested weak clinical signs of respiratory distress associated to lesions, among others, of PNP and interstitial pneumonia. Secondly, we needed to determine if the causal virus was still persisting in the farm four months after the conclusion of clinical signs and, whether or not, it could still represent a risk for replacement animals or seedstock sold to healthy farms. These objectives were met by a study on healthy sentinel pigs serving as indicators of the infection status with better accuracy than conventional laboratory submissions of native animals or contact experiments in isolated quarters.

The results demonstrated the principal role of PRRSV in the acute (symptomatic) and chronic (asymptomatic) phases of this syndrome, thus indicating sub-clinical persistence of the infection in some individuals by a fully pathogenic strain of PRRSV. Monitoring of sentinel pigs as long as 42 days PE showed that the infectious process was not necessarily accompanied by evidence of a severe clinical disease. Respiratory discomfort was not accompanied by anorexia and dullness which are usually the hallmark of a systemic disease. Nevertheless, fever was a prominent clinical manifestation as it was usually severe and prolonged, and was not related to secondary invaders.

Microscopic lesions were neither severe nor specific thus confirming the necessity for complementary means of identification. None of the sentinel pigs developed lesions of PNP, consistent with failure to find this condition in pigs euthanized six months earlier when clinical respiratory illness was present in the farm. Moreover, the involvement of an atypical type A influenza virus remaining unconfirmed by both virological and serological investigations. The demonstration that growing pigs were already seropositive to PRRSV nine months before the occurrence of reproductive problems on the farm, substantiate recent histological findings by other authors that more virulent strains of PRRSV may also be responsible for the development of PNP-like lesions (28). Indeed, hyperplasia of pneumocytes type II, as well as the presence of necrotic inflammatory cells and aggregates of proteinaceous membranes in the alveolar lumen and septae are microscopic features of PNP that have also been observed in lung sections of pigs experimentally- or naturally-infected with distinct PRRSV isolates (8,28, 29). Microscopic lesions of proliferative interstitial pneumonia characterized mainly by type II pneumocytic proliferation, syncytial cell formation and lesions of myocarditis have also been described in experimentally-inoculated piglets that showed seroconversion to PRRSV (28,29). Variations in the pathogenicity of various isolates of PRRSV have been suggested to explain the severity of the lesions described by different authors, as well as the capability of the virus to establish persistent infection (12,19, 30–32). Using the immunogold silver staining (IGSS) method, a recent retrospective study of 38 spontaneous cases of PNP in Québec pig farms tended to correlate frequent association between PRRSV and PNP in 73.7% of cases diagnosed by histopathology whereas only 2.6% of the PNP-affected lungs reacted positively with SIV (33). It appears that differentiation between these viral infections on the basis of histopathological findings alone is more difficult than it was initially reported (2,3,34). The development of reliable, specific and sensitive diagnostic tools should better clarify the relationship between these two new respiratory infections in pigs.

The infection of sentinel pigs by PRRSV was accompanied by a prolonged viraemia despite the presence of high levels of circulating antibodies. Furthermore, experimental inoculation studies of pregnant sows with a PRRSV strain isolated four months after the disappearance of significant clinical signs in the farm, confirmed that the persistent strain was still highly virulent. Thus, it appears that the virus can persist in some pigs, in different locations, despite high levels of antibodies.

It has been reported that anti-PRRSV antibodies detected by immunohistochemical techniques, such as indirect immunofluorescence and immunoperoxydase, have a limited *in vitro* virus neutralizing activity (7,30,35). Accordingly, only three of the sentinel pigs placed in barn A developed anti-PRRSV neutralizing antibodies, significant antibody titers were detected only after 35 days PE. The protective role of neutralizing antibodies against PRRSV reinfection needs to be demonstrated. Meanwhile, the interpretation of PRRSV serology and the relationship of serologically positive animals to clinical disease must be approached with caution. The detection of a specific humoral response to PRRSV should be considered as an indicator of exposure to the virus; positive serology does not verify disease and does not reflect the protective status of the animals (11,19,30). Indeed, the two sentinel pigs which were placed in the neighboring barn B (clinically healthy) also developed PRRSV infection, indicating that the virus was circulating in this barn, despite the absence of a clinical disease.

Studies are currently in progress in our laboratory to characterize the antigenic determinants of the virus. The development of diagnostic tests that would distinguish between the types of anti-PRRSV antibodies in the serum of infected pigs may predict the degree of protection and risk of disseminating the virus.

Our study offers no conclusive explanation for the presence of PRRSV infection in the farm nine months before the onset of clinical signs. This may have different explanations: 1), variations in pathogenicity or tropism of viral strains for the reproductive and respiratory systems; 2), they may have been repopulated in 1987 with infected pigs with protective immunity which waned during gradual replacement of the adult herd; 3), a delay in transmission of infection between grower-finisher and adult sections; and 4), triggering fac-

tors of unknown nature. Furthermore, it has been recently demonstrated that piglets from infected sows may carry the virus for a long period despite the presence of high levels of maternal antibodies. Experimentally, infected animals may excrete the virus up to 17 weeks after the disappearance of clinical signs, or 15 weeks after seroconversion. It has been demonstrated that healthy carriers may shed the virus after being submitted to stress or after immunodepression; such situations can be observed during the farrowing and weaning periods (30).

This study led to a policy of determining the PRRS serological status of seedstock producers and their potential clients before a sale. In conclusion, the results are in agreement with previous findings that PRRSV may persist in different age groups and locations for up to four months, despite absence of clinical signs on a farm for a significant period, and may therefore be transmitted by contact to replacement animals or via seedstock sold to uninfected farms.

ACKNOWLEDGMENTS

The authors thank Ms. Louise Wilson, Ms. Nicole Sawyer and Ms. Guylaine Morin for technical assistance. We also thank the following veterinarians and researchers from the Ministère de l'Agriculture, des Pêcheries et de l'Alimentation du Québec and the Laboratoire d'Hygiène Vétérinaire, Agriculture Canada, for their involvement in the experimental inoculation studies, and for the histopathological and serological analyses: Dr. Ronald Magar, Dr. André Gagnon, Dr. Pierre Helie and M. Robert Assaf. The collaboration of Drs. D.A. Benfield and E. Nelson, South Dakota State University, in providing the monoclonal antibodies to the ATCC-2332 strain of PRRS virus, was greatly appreciated. We also thank Dr. J. Kwang, U.S. Meat Animal Research Center, Clay Center, Nebraska, for providing the MARC-145 cells. The animals used for experimental inoculation studies were provided by Genetiporc Inc., St-Bernard de Beauce-Nord, Québec. We would also like to thank Drs. Robert Charette and Maurice Bessette, Clinique Vétérinaire St-Thomas d'Acquin, Québec, for providing data and specimens for initiating this study.

REFERENCES

1. **BILODEAU R, DEA S, MARTINEAU GP, SAUVAGEAU R.** Porcine reproductive and respiratory syndrome in Quebec. Vet Rec 1991; 129: 102–103.
2. **MORIN M, ROBINSON Y.** Causes of mystery swine disease. Can Vet J 1992; 33: 6.
3. **MORIN M, GIRARD C, ELAZHARY Y, FAJARDO R, DROLET R, LAGACE A.** Severe proliferative and necrotizing pneumonia: A newly recognized disease. Can Vet J 1990; 31: 837–839.
4. **WENSVOORT G, TERPSTRA C, POL JMA, TER LAAK EA, BLOEMRAAD M, DE KLUYVER EP, KRAGTEN C, VAN BUITEN L, DEN BESTEN A, WAGENAAR F, BROEKHUIJSEN JM, MOONEN PLJM, ZETSTRA T, DE BOER EA, TIBBEN HJ, DE JOG MF, VAN'T VELD P, GROENLAND GJR, VAN GENNEP JA, VOETS MT, VERHEIJDEN JHM, BRAAMSKAMP J.** Mystery swine disease in The Netherlands: the isolation of Lelystad virus. Vet Q 1991; 13: 121–130.
5. **TERPSTRA C, WENSVOORT G, POL JMA.** Experimental reproduction of porcine epidemic abortion and respiratory syndrome (mystery swine disease) by infection with Lelystad virus: Koch's postulates fulfilled. Vet Q 1991; 13: 131–136.
6. **WENSVOORT G, KLUYVER EP, POL JMA, WAGENAAR F, MOORMANN RJM, HULST MM, BLOEMRAAD R, DEN BESTEN A, ZETSTRA T, TERPSTRA C.** Lelystad virus, the cause of porcine epidemic abortion and respiratory syndrome: a review of mystery swine disease research at Lelystad. Vet Microbiol 1992; 33: 185–193.
7. **CHRISTIANSON WT, COLLINS JE, BENFIELD DA, HARRIS L, GORCYCA DE, CHLADEK DW, MORRISON RB, JOO HS.** Experimental reproduction of swine infertility and respiratory syndrome in pregnant sows. Am J Vet Res 1991; 53: 485–488.
8. **COLLINS JE, BENFIELD DA, CHRISTIANSON WT, HARRIS L, HENNINGS JC, SHAW DP, GOYAL SM, McCULLOUGH S, MORRISON RB, JOO HS, GORCYCA D, CHLADEK D.** Isolation of swine infertility and respiratory syndrome virus (isolate ATCC VR-2332) in North America and experimental reproduction of the disease in gnotobiotic pigs. J Vet Diagn Invest 1992; 4: 117–126.
9. **YOON IN J, JOO HS, CHRISTIANSON WT, KIM HS, COLLINS JE, CARLSON JH, DEE SA.** Isolation of a cytopathic virus from weak pigs on farms with a history of swine infertility and respiratory syndrome. J Vet Diagn Invest 1992; 4: 139–143.
10. **DEA S, BILODEAU R, ATHANASSIOUS R, SAUVAGEAU R, MARTINEAU GP.** Swine reproductive and respiratory syndrome in Quebec: Isolation of an enveloped virus serologically-related to Lelystad virus. Can Vet J 1992; 33: 801–808.
11. **YOON IN J, JOO HS, CHRISTIANSON WT, KIM HS, COLLINS JE, MORRISON RB.** An indirect fluorescent antibody test for the detection of antibody to swine infertility and respiratory syndrome virus in swine sera. J Vet Diagn Invest 1992; 4: 144–147.
12. **CHO SH, FREESE WR, YOON IJ, TRIGO AV, JOO HS.** Seroprevalence of indirect fluorescent antibody to porcine reproductive and respiratory syndrome virus in selected swine herds. J Vet Diagn Invest 1993; 5: 259–260.
13. **DEA S, BILODEAU R, SAUVAGEAU R, MONTPETIT C, MARTINEAU GP.** Antigenic variant of swine influenza virus causing proliferative and necrotizing pneumonia in pigs. J Vet Diagn Invest 1992; 4: 380–392.
14. **GIRARD C, MORIN M, ELAZHARY Y.** Experimentally-induced porcine proliferative and necrotising pneumonia with an influenza A virus. Vet Rec 1992; 130: 206–207.
15. **DEA S, BILODEAU R, ATHANASIOUS R, SAUVAGEAU RA, MARTINEAU GP.** Porcine reproductive and respiratory syndrome in Quebec: pathological and virological aspects. ASSP Newsletter 1992; 4: 3–7.
16. **KEFFABER KK.** Reproductive failure of unknown etiology. AASP Newsletter 1989; 1: 1–9.
17. **POLSON DD, MARSCH WE, DIAL GD.** Financial implication of mystery swine disease. Proceedings Mystery Swine Disease Meeting; Livestock Conservation Institute, Denver, Colorado, 1990: 8–28.
18. **GALINA L, PIJOAN C, SITJAR M, ROSSOW K, CHRISTIANSON W, COLLINS J.** Interaction between SIRS virus and *Streptococcus suis*. AASP Newsletter 1992; 4: 21–22.
19. **BAUTISTA EM, GOYAL SM, YOON IJ, JOO HS, COLLINS JE.** Comparison of porcine alveolar macrophages and CL 2621 for the detection of porcine reproductive and respiratory syndrome (PRRS) virus and anti-PRRS antibody. J Vet Diagn Invest 1993; 5: 163–165.
20. **MOLITOR T, LEITNER G, CHOI C, RISDAHL J, ROSSOW K, COLLINS J.** Modulation of host immune responses by SIRS virus. AASP Newsletter 1992; 4: 27–28.
21. **ZHOU Y, BARGHUSEN S, CHOI C, ROSSOW K, COLLINS J, LABER J, MOLITOR T, MURTAUGH M.** Effect of SIRS virus infection in leukocyte populations in the peripheral blood and on cytokine exprerssion in alveolar macrophages of growing pigs. AASP Newsletter 1992; 4: 28.
22. **JOO HS, DONALDSON-WOOD CR, JOHNSON RH.** Observation on the pathogenesis of porcine parvovirus infection. Arch Virol 1976; 51: 123–129.

23. **DEA S, BILODEAU R, SAUVAGEAU R, MARTINEAU GP.** Outbreaks in Quebec pig farms of respiratory and reproductive problems associated with encephalomyocarditis virus. J Vet Diagn Invest 1991; 3: 275–282.
24. **JABRANE A, ELAZHARY Y, TALBOT BG, ETHIER R, DUBUC C, ASSAF R.** Porcine respiratory coronavirus in Quebec: Serological studies using a competitive inhibition enzyme-linked immunosorbent assay. Can Vet J 1992; 33: 727–733.
25. **KIM HS, KWANG J, YOON IJ, JOO HS, FREY ML.** Enhanced replication of porcine reproductive and respiratory syndrome (PRRS) virus in a homogeneous subpopulation of MA-104 cell line. Arch Virol 1993; 133: 477–483.
26. **DEA S, ELAZHARY MASY.** Cultivation of a porcine adenovirus in porcine thyroid cells cultures. Cornell Vet 1984; 74: 207–217.
27. **DEA S, ELAZHARY MASY, MARTINEAU GP, VAILLANCOURT J.** Parvovirus-like particles associated with diarrhea in unweaned piglets. Can J Comp Med 1985; 49: 343–345.
28. **HALBUR PG, PAUL PS, ANDREWS JJ, SANDERSON TP, ROSS RF, SCHWARTZ KJ, FREY ML, ERICKSON BJ, HILL T, HOFFMAN LJ.** Experimental transmission of an apparent viral pneumonia in conventinal and gnotobiotic pigs. Vet Rec 1993; 132: 263–266.
29. **MAGAR R, LAROCHELLE R, ROBINSON Y, DUBUC C.** Immunohistochemical detection of porcine reproductive and respiratory syndrome virus using colloidal gold. Can J Vet Res 1993; 57: 300–304.
30. **ALBINA E, MADEC F, CARIOLET R, PABOEUF F, HUTET E, PANSART JF, KERANFLEC'H A, LEFORBAN Y.** Le syndrome dysgénésique et respiratoire du porc (SDRP): Persistance du virus chez l'animal et dans les élevages infectés. J Rech Porcines en France 1993; 25: 363–372.
31. **MARDASSI H, ATHANASSIOUS R, MOUNIR S, DEA S.** Porcine reproductive and respiratory syndrome virus: Morphological, biochemical and serological characteristics of Quebec isolates associated with acute and chronic outbreaks of porcine reproductive and respiratory syndrome. Can J Vet Res 1994; 58: 55–64.
32. **HALBUR PG, PAUL PS, MENG X, HAGEMOSER W, LUM M.** Variable pathogenicity of nine porcine reproductive and respiratory syndrome virus (PRRSV) isolates. Abstract 74th Conference Research Workers in Animal Diseases. Chicago, 1993.
33. **LAROCHELLE R, HELIE P, SAUVAGEAU R, MAGAR R.** Immunohistochemical detection of swine influenza virus and porcine reproductive and respiratory syndrome virus in porcine proliferative and necrotizing pneumonia cases. Abstract 74th Conference of Research Workers in Animal Diseases. Chicago, 1993.
34. **COLLINS JE.** Newly recognized respiratory syndromes in North American swine herds. AASP Newsletter 1991; 3: 9–11.
35. **MORRISON RB, COLLINS JE, HARRIS L, CHRISTIANSON WT, BENFIELD DA, CHLADEK DW, GORCYCA DE, JOO HS.** Serologic evidence incriminating a recently isolated virus (ATCC VR-2332) as the cause of swine infertility and respiratory syndrome (SIRS). J Vet Diagn Invest 1992; 4: 186–188.