

Antibody Production and Blastogenic Response in Pigs Experimentally Infected with Porcine Reproductive and Respiratory Syndrome Virus

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ABSTRACT

Seven five-week-old piglets were infected intranasally with 10^5 TCID₅₀ of porcine reproductive and respiratory syndrome (PRRS) virus strain IAF.exp91. All virus-exposed pigs developed fever, labored abdominal breathing, conjunctivitis, and lymph node enlargement within the first 96 h postexposure (PE), which continued to d 10 to 14 PE. Two pigs that were necropsied at d 7 and 10 PE had diffuse interstitial pneumonitis, cardiopathy and lymphadenopathy. All 5 remaining pigs produced serum IgM and IgG antibodies against PRRS virus by 7 or 14 days PE, as demonstrated by indirect immunofluorescence. This corresponded with the capability of isolating the virus from serum d 7 to d 49 or d 63 PE. Low serum neutralizing antibody titers were detected in 3 of the virus-exposed pigs by 35 days PE. A transient episode of diminished proliferative response of peripheral blood lymphocytes to mitogens phytohemagglutinin (PHA) and concanavalin A (Con A) was observed in the virus-exposed pigs at d 3 PE. However, *in vitro* spontaneous uptake of [³H]-thymidine was significantly increased in lymphocyte cultures of the same pigs at d 7 or d 14 PE. These results suggest polyclonal activation of peripheral blood lymphocytes.

RÉSUMÉ

Sept porcelets âgés de cinq semaines ont été infectés par voie intranasale avec 10^5 DECP₅₀ de la

souche IAF.exp91 du virus du syndrome reproducteur et respiratoire du porc (SRRP). Tous les porcelets exposés au virus ont présenté une hyperthermie, une respiration abdominale profonde, une conjonctivite et une hypertrophie des ganglions lymphatiques dans les 96 heures suivant l'inoculation virale. Ces signes cliniques ont persisté jusqu'au jour 10 ou 14 postexposition (PE) au virus SRRP. Des lésions de pneumonie interstitielle diffuse, de cardiopathie et de lymphadénopathie ont été observées chez les deux porcelets nécropsiés aux jours 7 et 10 PE. Une analyse sérologique réalisée chez les cinq autres porcelets en utilisant un test d'immunofluorescence indirecte a démontré la présence d'anticorps de type IgM et IgG au jour 7 ou 14 PE. Une correspondance a été notée entre la détection de ces anticorps et la capacité d'isoler le virus dans le sérum de ces animaux à partir du jour 7 PE jusqu'au jour 49 ou 63 PE. Des titres peu élevés d'anticorps sériques neutralisants spécifiques du virus SRRP ont été détectés chez trois des cinq porcelets exposés au virus à partir du jour 35 PE. Une diminution transitoire de l'activité blastogénique des lymphocytes du sang périphérique aux agents mitogènes phytohémagglutinine (PHA) et concanavaline A (ConA) ont été observées chez les porcelets exposés au virus au jour 3 PE. Par contre, une augmentation de l'incorporation spontanée de la thymidine tritiée *in vitro* a été observée dans les cultures lymphocytaires originant des mêmes

porcelets aux jours 7 ou 14 PE. Ces résultats suggèrent une activation polyclonale des lymphocytes du sang périphérique chez les animaux exposés au virus SRRP.

(Traduit par docteur Serge Messier)

The disease caused by the porcine reproductive and respiratory syndrome (PRRS) virus, an arterivirus of the family *Togaviridae* (1,2), is characterized by reproductive disorders (mummified, stillborn, or weak piglets) in sows of any parity, increased preweaning mortality, and respiratory disorders in pigs of all ages (3,4). Experimental reproduction of the disease has been successful in sows at mid- and late-term gestation and in fetuses (5-7). The disease has also been reproduced in piglets of different ages, with the appearance of characteristic clinical signs of fever and dyspnea and lesions of interstitial pneumonitis (3,4,8-10). In these studies, the effect of PRRS virus on lymphocyte functional reactivity was not documented.

The biological hallmark of the PRRS virus is that it infects and replicates (perhaps exclusively) in alveolar macrophages (3,4). Moreover, the virus appears to persist for a long time, despite the ability of the host to mount a virus-specific immune response (11-14). Because PRRS virus infects macrophages, a primary barrier cell population of the host defense system, appears to persist for a long time within the infected host, and pigs of PRRS virus-positive herds appear more susceptible to secondary infections (4,15), one may suspect the PRRS virus for

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TABLE 1. Kinetics of serum antibody titers to PRRS virus in infected pigs as measured by indirect immunofluorescence assay (IIFA)^a

Pig number	Days post virus exposure								
	0	3	7	14	21	28	35	49	63
4	<16 ^b (<16) ^c	<16 (<16)	<16 (32)	64 (512)	256 (128)	512 (64)	512 (32)	512 (NT) ^d	512 (NT)
5	<16 (<16)	<16 (<16)	<16 (128)	32 (512)	256 (128)	512 (32)	>1024 (32)	>1024 (<16)	>1024 (<16)
6	<16 (<16)	<16 (<16)	<16 (128)	64 (>1024)	512 (512)	512 (128)	512 (128)	>1024 (32)	>1024 (<16)
7	<16 (<16)	<16 (<16)	32 (128)	128 (512)	512 (128)	>1024 (128)	>1024 (64)	>1024 (<16)	>1024 (<16)
11	<16 (<16)	<16 (<16)	32 (128)	32 (>1024)	>1024 (128)	>1024 (128)	>1024 (64)	>1024 (<16)	>1024 (<16)

^a Antibody titer to PRRS virus expressed as the reciprocal of the highest serum dilution positive by IIFA using IAF.exp91 PRRS virus-infected alveolar macrophages as indicator cells

^b PRRS-specific IgG antibody titers

^c The number within parentheses indicate PRRS virus-specific IgM antibody titers

^d NT: not tested

causing dysfunction or suppression of the host immune system.

In this report, we describe aspects of the immune response of specific-pathogen-free (SPF) pigs to PRRS virus. The study included 13 pigs that were purchased from a herd free of atrophic rhinitis, *Mycoplasma hyopneumoniae*, *Serpulina hyodysenteriae*, *Actinobacillus pleuropneumoniae*, and *Salmonella* spp. The source herd was also seronegative for antibodies to PRRS virus, transmissible gastroenteritis virus (TGEV), porcine parvovirus (PPV), encephalomyocarditis virus (EMCV), swine influenza virus (SIV), and porcine hemagglutinating encephalomyelitis virus (HEV), and to bovine viral diarrhea virus (BVDV) (16). The pigs were 3.5 wk old when they were delivered to our laboratory, randomly placed into 2 experimental groups (control pig nos 1, 2, 3, 8, 9, and 10, and virus-exposed pig nos 4, 5, 6, 7, 11, 12, and 13), and separated by group in isolation facilities. The pigs were maintained on an appropriate commercial diet containing no antibiotics. At 5 wk old (i.e., after 10 d to adapt to their new environment), they were exposed intranasally to 5 mL of cell culture fluid that either was free of PRRS virus (control pigs) or contained 10⁶ median cell culture infective doses (TCID₅₀) (12th virus subculture) of PRRS virus (virus-exposed pigs).

All pigs were observed daily for clinical signs throughout the experiment. Rectal temperatures of both control and virus-exposed pigs were taken 3 d before virus exposure, on the day of virus exposure (d 0), and

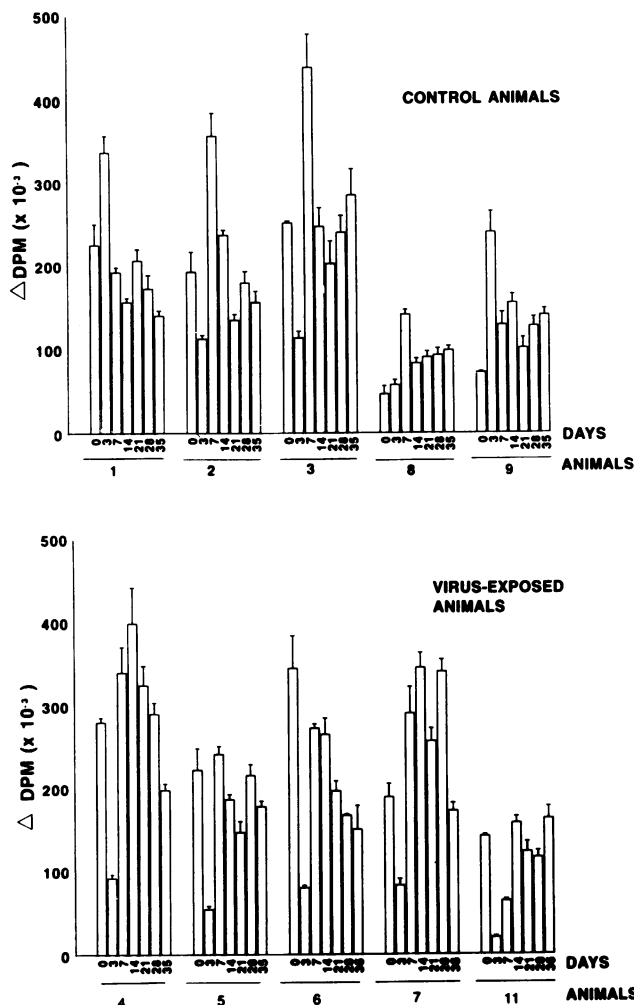
daily thereafter until the end of the experiment on d 63. Blood samples were collected from each pig on d 0 (just before exposure), d 3, 7, 14, 21, 28, 35, 49, and 63 postexposure (PE). Sera were tested for antibody to PRRS virus by indirect immunofluorescence assay (IIFA) in primary porcine alveolar macrophages (PAM) (13). All IIFA's were run in duplicate with fluorescein isothiocyanate-labeled conjugates prepared from rabbit antiserum for either porcine IgM (Cedarlane Laboratories Ltd, Hornby, Ontario) or porcine IgG (ICN Biochemicals Canada Ltd, Mississauga, Ontario). The IIFA antibody titers were expressed as the highest dilution demonstrating positive cytoplasmic fluorescence (13). Sera were also tested for PRRS virus-specific antibodies by virus neutralization (VN) in MARC-145 cells (14,17). The neutralizing antibody titer of each serum was expressed as the highest serum dilution neutralizing 100 TCID₅₀ of PRRS virus (14). Finally, sera were tested for infectious PRRS virus by virus isolation and identification in PAM (16). Peripheral blood lymphocytes were tested for their blastogenic response to mitogens phytohemagglutinin (PHA) and concanavalin A (Con A) based on a published procedure (18). PHA (Sigma Chemical, St-Louis, Missouri), or Con A (Pharmacia, Dorval, Québec) were used at optimal concentrations of 1.25 µg per cell culture (2 × 10⁵ cells per well culture, 4 wells per mitogen). The data were expressed in disintegrations per min (dpm) and the delta dpm was calculated as described elsewhere (18).

Lymphocyte proliferative activity differences over time were tested by repeated measure analysis of variance using SAS (Cary, North Carolina) (19). Differences between control and virus-infected animals were identified by significant interaction effects between time and treatment (virus infection). Significant effects were pinpointed with single degree-of-freedom contrasts between d 0 (before virus infection) and subsequent dates after virus infection. The Quebec IAF-exp91 strain of PRRS virus that had been propagated in primary cultures of PAM as previously described (16) was used for intranasal exposure of pigs, for IIFA, and for virus isolation. The IAF.Klop strain of PRRS virus that has been adapted for MARC-145 cells (20) was used for VN tests.

All pigs were clinically normal upon arrival in the isolation facilities and for the entire adaptation period before virus exposure. The first clinical abnormality found in the PRRS virus-exposed pigs was an elevation in body temperature ranging from 40 to 41.6°C at day 1 PE. This was followed by a 2nd episode of hyperthermia (40 to 42°C) at 72 h PE, after which time the temperatures in the virus-exposed animals were in the normal range (38.1 to 38.8°C) to the end of the 63-d observation period. Control pigs had body temperatures within the normal range throughout the experiment. Signs of periorbital edema and mild conjunctivitis accompanied by laboured abdominal breathing and lymph node enlargement in the pelvic region were generally observed in virus-exposed pigs beginning at d 3 PE and continuing to d 10 to 14 PE. No clinical signs were observed in the virus-exposed pigs from d 15 PE. All the controls remain clinically normal throughout the experiment.

Two of the virus-exposed pigs (nos 12 and 13), with the clinical signs described above, were euthanized at d 7 and 10 PE. Gross lesions seen in these pigs were similar and were mainly confined to the respiratory tract. The lungs were partly collapsed and had a discoloration of the surface with a greyish and glossy appearance and the presence of petechiae dispersed through the lung surface. The ventral pulmonary lobes were somewhat hyperemic and the mediastinal

A. PHA-STIMULATED CELL CULTURES



B. CON A-STIMULATED CELL CULTURES

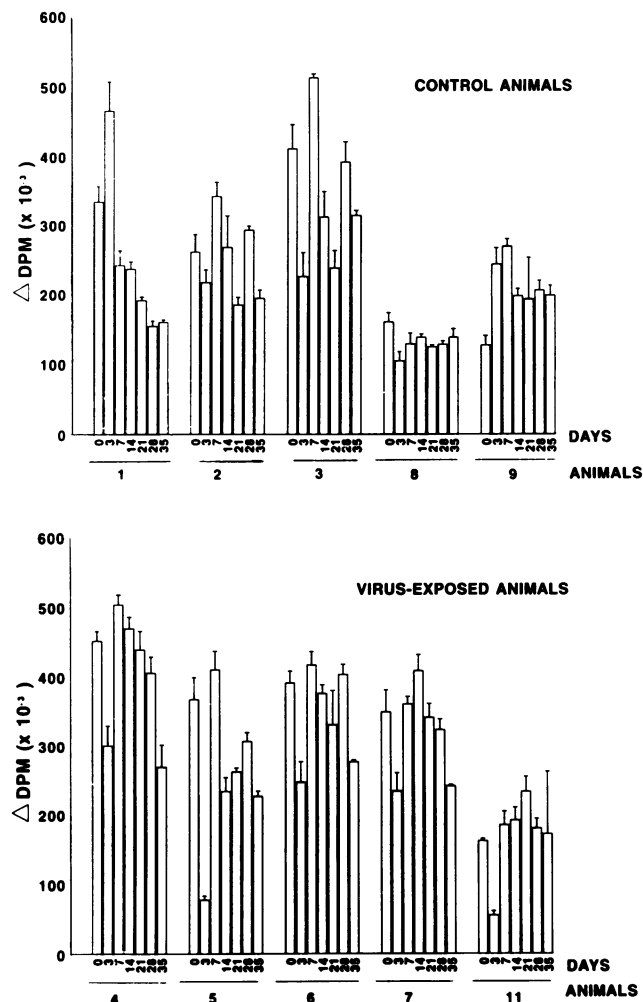


Figure 1. In vitro proliferative response to mitogens phytohemagglutinin (PHA) (1A) or concanavalin A (Con A) (1B) of peripheral blood lymphocytes isolated from PRRS virus-exposed and control pigs.

lymph nodes were distended and edematous. Paint brush-like hemorrhagic foci were also seen at the myocardial surface. The popliteal lymph nodes were enlarged. Microscopically, pulmonary lesions consisted of a proliferative interstitial pneumonitis characterized by hyperplasia of type II pneumocytes accompanied by alveolar septal infiltration by macrophages and a few lymphocytes, with the occasional presence of necrotic cells in the alveolar lumen and necrotic foci within the pulmonary parenchyma. A moderate peribronchiolar and perivascular lympho-histiocytic cell infiltration was also observed. Lesions of lymphomonocytic myocarditis were also encountered in the heart ventricles. Hyperplasia of the lymphoid follicles was observed in the popliteal lymph nodes and in the spleen. No significant gross or histologic lesions

were observed in 1 control pig (no 10) euthanized on d 10 after beginning the experiment. Virus could be rescued in PAM cells from the lungs of the 2 virus-exposed pigs.

All other pigs exposed to the virus (nos 4, 5, 6, 7 and 11) developed significant (≥ 16) PRRS virus-specific antibody titers in their serum by 7 to 14 d PE as determined by IIFA (Table I). The IgM serum antibodies reached maximal titers of 512 to >1024 at d 14 PE; low IgM antibody titers of 32 to 128 were observed at d 35 PE. All pigs were PRRS virus-specific IgM negative by d 49 or 63 PE. In contrast, levels of IgG serum antibodies increased over time to reach maximal IIFA antibody titers of 512 to >1024 by d 21 to 49 PE and persisted to the end of the 63-d period. On the other hand, low neutralizing antibody titers (16 or 32) were

detected in only 3 virus-exposed pigs (nos 4, 5, and 11) by d 28 or 35 PE, and remained at that level to the end of the 63-d period. No PRRS virus-specific IIFA or neutralizing antibodies were detected in control pigs. Virus could be rescued in PAM cells from serum of all virus-exposed animals from d 7 to 49 PE. In 2 infected pigs (nos 7 and 11), virus was isolated from the serum at d 63 PE, in spite of the presence of serum PRRS virus-specific neutralizing antibodies.

The kinetics of the in vitro proliferative response of blood lymphocytes to mitogens in each tested pig were followed during the first 35 d PE (Fig. 1). Although the blastogenic activity was individual and time-variable, transient decreases of the lymphoproliferative responses to mitogens PHA (Fig. 1A) and Con A (Fig. 1B) were observed in all pigs exposed to

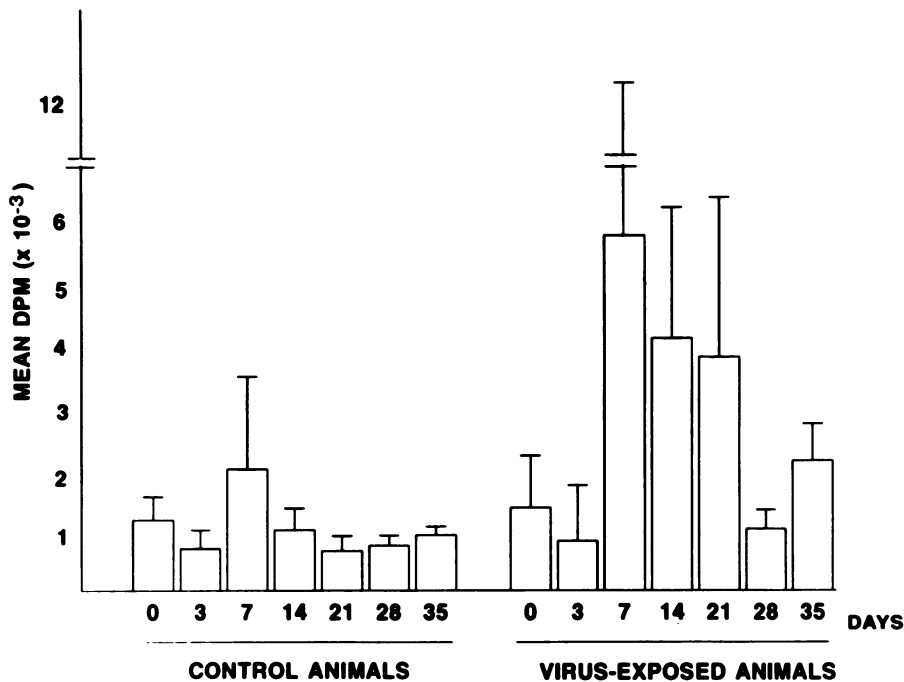


Figure 2. In vitro spontaneous [³H]-thymidine incorporation by peripheral blood lymphocyte cultures isolated from PRRS virus-exposed and control pigs.

the PRRS virus at d 3 PE. One of the 5 virus-exposed pigs (no 11) was also showing a diminished lymphocyte activity to PHA at d 7 PE. A slight decrease in the lymphocyte activity to mitogens was also seen in 2 control pigs (nos 2 and 3) at d 3 after beginning the experiment, but was less intense than in the virus-exposed animals. Statistical analysis of the results over time showed a significant difference between control and virus-exposed animals, with a diminution of the lymphocyte activity to PHA ($P < 0.02$) or Con A ($P < 0.053$) at d 3 PE in the virus-exposed animals when compared to the controls.

The kinetics of the in vitro spontaneous blastogenic activity (mitogen nonstimulated control lymphocyte cultures) in each group of virus-exposed and control pigs were also investigated (Fig. 2). The data obtained for these lymphocyte cultures were less variable from pig to pig than those obtained for the mitogen-stimulated cultures, with comparable mean dpm of 1159 and 1326 for the control and virus-exposed groups of pigs, respectively, before virus exposure (d 0). A noticeable enhancement of the in vitro lymphocyte spontaneous blastogenic activity was observed beginning at d 7 PE and continued to be significantly increased ($P < 0.05$) at d 14 PE.

Day 21 values were still elevated in the virus-exposed group of pigs, but the difference was not significant ($P < 0.08$). Day 35 values were also elevated in the virus-exposed animals, but tended to return to d 0 values.

The results of this study demonstrate that PRRS virus-exposed pigs show the typical clinical signs and lesions associated with respiratory disorders, as has been previously described (4,8,10). Pigs exposed to the PRRS virus specifically seroconverted at d 7 or d 14 PE, as determined by IIFA. This is consistent with previous findings in piglets of different ages infected with various PRRS virus isolates (10,13,14). Paradoxically, the virus could be routinely isolated from sera of the virus-exposed pigs from d 7 to 49 PE, and, in the case of 2 animals, to the end of the 63-d observation period. The correspondence between the onset of virus isolation and the 1st appearance of serum IIFA antibodies indicates that these PRRS virus-specific circulating antibodies are not effective in virus clearance, as has been reported by others (11, 12,14). This suggests that PRRS virus may persist in naturally or experimentally infected animals for a long period, despite high levels of circulating IIFA antibodies. This state of virus persistence may reflect the inability of

certain infected animals to mount a protective immune response. Indeed, 3 of the 5 experimentally infected pigs showed low levels of circulating neutralizing antibodies that appeared to not be predictive of virus clearance. This is in agreement with other studies where anti-PRRS virus antibodies detected by immunohistochemical techniques, such as IIFA or immunoperoxidase, had no or limited in vitro virus neutralizing activity (4,11,12,14), and did not reflect the protective status of the animals.

The levels of serum IIFA IgG antibodies increased over time to reach maximal titers by 21 to 49 d PE, at which time IgM antibody titers were declining. Moreover, IgG antibodies were still elevated at 63 d PE in all virus-exposed pigs, whereas IgM antibodies were no longer detectable. These results suggest that detection of IIFA IgM antibodies could reflect recent exposure to the PRRS virus. Detection of high serum IIFA IgG antibody titers indicates a previous infection, but does not indicate when the animal was infected. Indeed, IIFA IgG antibody titers are still elevated (>1280) in pigs 4 mo after PRRS virus exposure (14).

The infection of pigs with PRRS virus resulted in a diminished proliferative response of blood lymphocytes to both mitogens PHA and Con A. This effect was only observed at d 3 PE, and, in regard to its transient nature, was similar to the depressed T cell responses (as measured by an increased survival of tissue or tumor transplants, a reduced delayed-type hypersensitivity, or a reduced response of spleen cells to Con A) in mice during the acute phase of infection with the lactate dehydrogenase-elevating virus (LDV) (21–23). LDV is an agent of the arterivirus group related to PRRS virus, whose target cell is also the macrophage (23).

Significant enhancement of the in vitro proliferative activity of the control cell cultures (with no mitogen) was obtained with blood mononuclear cells isolated from the PRRS virus-exposed pigs. This effect was significant at d 14 PE, and tended to persist over time. Similar increased spontaneous [³H]-thymidine incorporation by spleen cells from mice infected with LDV was reported in vivo, as well as in vitro (22,24,25).

In mice, in contrast to the diminished cellular immune responses during the primary phase of LDV infection, humoral antibody responses to T-dependent and T-independent antigens are enhanced during the early phases of infection (21,23). This effect, which correlates with enhanced *in vitro* background proliferative activity of lymphocyte cultures, is believed to be related to the initiation of polyclonal activation of B cells and rapidly increased IgG_{2a} production caused by LDV infection (22,24–27). Whether such mechanisms of lymphocyte activation are present in PRRS virus-infected pigs remains to be investigated. However, an enhanced antibody production to T cell-dependent antigens (4,15), and the enhanced spontaneous [³H]-thymidine incorporation in lymphocytes isolated from our PRRS virus-infected pigs, might support this interpretation.

The effect of PRRS virus infection on the host immune system thus appears to be bivalent. In earlier phases of the infection, PRRS virus appears to cause a transient diminution of the proliferative activity of blood lymphocytes to mitogens as reported here and an impaired function of alveolar macrophages, as reported by others (4). This diminished immune reactivity appears to be rapidly overcome by the host since antibody production and specific cellular immune response to heterologous T cell-dependent antigens are normal, or even enhanced, in PRRS virus-exposed pigs (15). Whether or not the PRRS virus has a role in the recrudescence of bacterial secondary infections in PRRS virus-positive herds remains unanswered at this point.

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