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Porcine reproductive and respiratory syndrome virus (PRRSv) interaction with *Haemophilus parasuis*

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Abstract

The interaction of bacteria and virus has been well demonstrated in the pathogenesis of respiratory disease in swine. The interaction between porcine respiratory and reproductive syndrome virus (PRRSv) and *Haemophilus parasuis* has not been studied. We initiated studies to evaluate a possible effect of the PRRSv on the pathogenesis of polyserositis caused by *H. parasuis*. A group of 30 three week old piglets were distributed in 4 groups. Group I (10 pigs) was inoculated with PRRSv and *H. parasuis*. Group II (10 pigs) was inoculated with *H. parasuis* alone. Group III (5 pigs) was inoculated with virus alone and group IV (5 pigs) was inoculated with culture media. Lesions consisted of a severe fibrinous polyserositis affecting 7 of 10 animals in group II and a mild fibrinous pleuritis in 1 of 10 animals of group I. Three of ten animals dually infected with the two agents died during the course of the study. These animals had pulmonary congestion and focal lung hemorrhages. No other animals died from other groups. Group III and IV had no macroscopic lesions. Microscopically group III had interstitial pneumonia. Immunomodulating virus effect may explain the differences in terms of lesions severity between groups I and II. Septic shock was suspected as cause of sudden death. © 1997 Elsevier Science B.V.

Keywords: PRRSv; *H. parasuis*; Interaction; Polyserositis; Pneumonia; Immunomodulation; Septic shock

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1. Introduction

Glässer's disease caused by *Haemophilus parasuis* is manifested in conventional herds as fibrinous polyserositis in 5–8 week-old piglets as described by Nielsen and Danielson (1975). Stress conditions such as weaning or transport have been suggested as risk factors for the presentation of the disease (Menard and Moore, 1990). There has been a recent increase of fibrinous polyserositis outbreaks in the USA, possibly because of the high health status and immunological naive pigs produced using new technologies such as medicated early weaning (MEW) described by Wiseman et al., 1994. Alternatively, the increase in incidence may reflect widespread infection by PRRS virus, a putative immunosuppresive agent related to respiratory disease complex in swine (Done et al., 1992, Christianson and Joo, 1994). PRRSv has been suggested to increase the severity of secondary bacterial outbreaks increasing mortality and poor doing animals in nurseries and growers (Pijoan et al., 1994), with a major economical impact in the last five years (Collins et al., 1991a; Benfield et al., 1992; Kerkaert et al., 1994).

Field observations suggest an increase in atrophic rhinitis, polyserositis and bacterial meningitis in PRRS infected pigs (Collins, 1991b). PRRS virus infection may predispose pigs to secondary infections by damaging non-specific respiratory defenses through the destruction of alveolar macrophages that may be substituted by immature cells (Molitor, 1993) and by inducing inflammation in the nasal mucosa (Collins et al., 1992c; Galina, 1995; Rossow et al., 1995).

Experimentally PRRS has been shown to predispose pigs to *Streptococcus suis* meningitis (Galina et al., 1994a; Galina et al., 1994b). Infection of susceptible pigs with PRRS virus, followed 5 days later by intranasal challenge with *S. suis*, resulted in central nervous clinical signs and meningitis. Other workers have suggested interaction between PRRS virus and a low-virulence strain of *Actinobacillus pleuropneumoniae*, resulting in increased clinical signs and lesion severity (Wensvoort, 1995). Interaction between PRRS virus and other viruses has also been demonstrated. Pigs coinfected with porcine respiratory coronavirus (PRCV) and PRRS virus developed severe clinical signs (Van Rieth et al., 1994) whereas results were variable when pigs were coinfected with swine influenza virus and PRRS virus (Brun et al., 1994).

The objective of the present study was to evaluate the possible in vivo interaction between PRRS virus and *H. parasuis* in young pigs.

2. Materials and methods

2.1. Animals

Thirty piglets, between 9 and 12 days of age were obtained from five litters of a PRRS virus and *H. parasuis* seronegative herd. After arrival, piglets were maintained with a special antibiotic schedule for three consecutive days [1] while they were allowed to acclimate before the initiation of the experiments. Medication was withdrawn six days

[1] Littermilk, Land O'Lakes, St. Paul, MN.

Table 1
Experimental design: Group distribution and challenge of thirty 3 week old piglets

Group	Treatment	No. pigs/group	PRRSv inoculum	Hps inoculum
I	PRRSv-Hps	10	+	+
II	Hps	10	–	+
III	PRRSv	5	+	–
IV	Control	5	–	–

before the bacterial inoculation. Animals were housed in the College of Veterinary Medicine isolation facilities, following the procedures of the University of Minnesota Animal Care and Use Committee.

2.2. Viral inoculum

One ml of PRRSv strain VR-2332 with a titer of 10^5 TCID 50/ml (passage level 3) grown in CL 2621 cells was used for intranasal inoculation [2]. Characterization of this isolate has been previously described (Collins et al., 1992c).

2.3. Bacterial Inoculum

A strain of *Haemophilus parasuis* serotype 5 (strain, 29755) was used [3]. This strain has previously been shown to be virulent for SPF pigs (Rapp-Gabrielson and Gabrielson, 1992). Piglets were inoculated intratracheally with 1.0 ml of *H. parasuis* containing 10^7 CFU/ml. Briefly, after sedation with a xylacine [4] and ketamine [5] combination a laryngoscope was used to introduce an endotracheal tube [6]. Bacterial suspension was slowly administered during inspiration.

2.4. Experimental design

Thirty piglets were randomly assigned to two groups of 10 piglets each (groups I and II) and two groups of 5 piglets each (groups III and IV). An equal number of animals from each litter were allotted to every group. The groups were inoculated according to Table 1.

Virus inoculation in groups I and III was three days after arrival. Bacterial inoculation was on day 8, five days after the initial viral challenge. The timing of exposure was based on previous results (Molitor et al., 1992; Rossow et al., 1994) that showed a profound damage of alveolar macrophages on day 7 post-virus infection.

[2] Provided by Dr. Molitor, University of Minnesota, St. Paul, MN.
[3] Provided by Dr. Ross, Iowa State University, Ames, IA.
[4] Rompun, Milles, Agriculture Division Animal Health, Shawnee Mission, KS.
[5] Ketaset, Fort Dodge Laboratories, Fort Dodge, IA.
[6] Sheridan Catheter, Argyle, NY.

Rectal temperatures and clinical signs were recorded daily. Complete post-mortem examinations were made when piglets from each group either died or when their condition was determined critical based on the presence of two or more signs of recumbency, acute central nervous system signs and hypothermia at which time they were euthanized with 2 ml of intravenous injection of sodium pentabarbithal [7]. Animals without severe signs were euthanized five days after bacterial inoculation. Blood samples from all animals were collected on day of arrival, at the time of bacterial inoculation and at necropsy for serological measures of PRRSv antibody titer.

2.5. Pathology

Necropsy was performed on each animal. Samples from lung, trachea, nasal turbinates, heart, kidney, tonsil, thymus, spleen, ileum, liver and mediastinal, retropharyngeal, inguinal and mesenteric lymph nodes were collected. One lung and trachea were suspended in 10% neutral buffered formalin and perfused with the same fixative at 20 cm of pressure until the lung volume approximated thoracic limits as previously described by others (Rossow et al., 1995; Hayatdavoudi et al., 1980). The trachea was ligated with string and the lung was fixed for 48 h. Transverse sections of dorsal, cranial and middle lung lobes were collected for microscopic examination. All other tissues were fixed by immersion in formalin. Fixed samples were dehydrated, embedded in paraffin, sectioned at 4 mm and stained by hematoxylin–eosin.

2.6. Bacteriological isolation and identification

Tonsilar swabs collected on the day of arrival and post-mortem swabs of cerebro-spinal, ascitic and pericardic fluids, trachea, lung and joint were immediately plated on chocolate agar plates and blood agar plates with a nurse strain of *Staphylococcus aureus*. After 48 h of incubation at 37°C with 5% CO_2 isolated colonies were taken for further biochemical testing. Urease, NAD dependent growth, Gram stain and Levulinic acid tests were performed. Isolates were then subcultured on PPLO media. Samples were frozen at −20°C.

2.7. Serology

Serum samples were tested for anti-PRRSv antibodies by an inmmunofluorescent antibody test (IFA) using VR-2332 PRRSv infected CL 2621 cell monolayer as the antigen and a fluorescent labelled antispecies conjugate as the indirect stain (Yoon et al., 1992).

2.8. Statistical analysis

Repeated measures of variance were used for comparing rectal temperatures among groups. An additive linear model for categorical data was performed to analyze lesions and mortality. Significant difference was considered when $p < 0.05$.

[7] Beuthanasia D-special, Schering Plough Animal Health, Kenilworth, NJ.

3. Results

3.1. Clinical observations

There were no clinical signs following viral challenge. On the groups challenged with the virus (groups I and III) only a mild increase in rectal temperature was seen ($p > 0.05$).

After the *Haemophilus parasuis* inoculation (groups I and II), pigs developed central nervous signs such as padling, nystagmus and tremor. Pigs challenged with bacteria only (group II) were more severely affected since they presented more than one central nervous sign. There was a significant difference in mean rectal temperature among groups following bacterial challenge. Groups dually infected with PRRSV and *H. parasuis* (group I) or *H. parasuis* alone (group II) had higher mean temperatures than controls (group IV) or the group infected with virus only (group III) ($p < 0.05$) (Fig. 1). Over the days 4, 5, 6 and 7 the difference was most pronounced one day after bacterial challenge (day 6). The group infected only with *H. parasuis* (group II) had a higher mean temperature than the other groups ($p < 0.05$).

Sudden death (3/10) was observed only in the group coinfected with virus and bacteria (group I). The mortality between groups I and II was different ($p < 0.1$).

3.2. Pathology

Animals challenged only with bacteria (Group II) had more severe and generalized polyserositis, characterized by large deposits of fibrin and fibrinous adherences of lung to thoracic cavity. Animals dually challenged with virus and bacteria (group I) had less severe and more localized lesions. The group inoculated with PRRSV only (group III) or the controls (group IV) had no macroscopic lesions.

Microscopic results are summmarized in Table 2. In Group I only, one animal had fibrinous pleuritis. In group II, 7 of 10 animals had localized or generalized fibrinous polyserositis (Table 3). Fibrinous serositis was characterized by the presence of fibrin

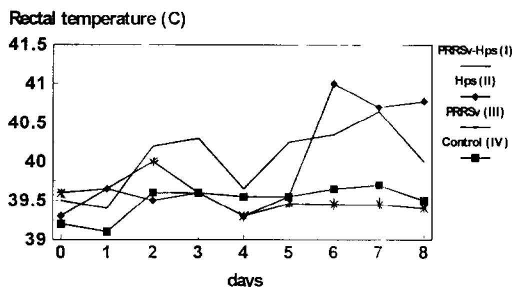

Fig. 1. Mean rectal temperature of piglets experimentally infected with PRRSv and *H. parasuis* (group I), *H. parasuis* (group II), PRRSv (group III) and placebo (group IV). Viral challenge was at day 0 followed by bacterial challenge on day 5. Group infected only with *H. parasuis* (group II) had a higher mean temperature than the other groups.

Table 2
Microscopic lesions observed in serosas and lungs of pigs experimentally infected with PRRSv and Hps

Group	Serositis	Interstitial pneumonia	Bronchopneumonia
I	1/10	10/10	2/10
II	7/10	0/10	4/10
III	0/5	4/5	0/5
IV	0/5	0/5	0/5

Table 3
Distribution of microscopic lesions observed in different serosas of pigs experimentally infected with PRRSv and Hps (group I) and Hps (group II)

Group	Pleuritis	Pericarditis	Peritonitis	Meningitis
I	1/10	0/10	0/10	4/10
II	7/10	2/10	4/10	5/10

and variable amounts of inflammatory cells, consisting of a mixture of monocytes, lymphocytes and polymorphonuclear neutrophils (PMNN) (Fig. 2).

Four of 10 animals in group I and 5 of 10 animals in group II had meningitis, characterized by fibrinous exudate with macrophages and PMN cells (Fig. 3). No

Fig. 2. Lung pleura of a pig 3 days after treatment with *H. parasuis*. Note the presence of fibrin and mixture of inflammatory cells lining the pleura. HE 250×.

Fig. 3. Meninges of a pig 2 days after exposure to *H. parasuis*. Note the presence of inflammatory cells in the subarachoid space of the leptomeninges. HE 250×.

significant differences were observed between meningitis lesions found in groups I and II ($p > 0.05$).

Interstitial pneumonia characterized by a mild macrophage and lymphocyte cell infiltration and thickened alveolar septa was present in all groups infected with the virus (group I and III) (Fig. 4). Catharral–purulent bronchopneumonia consisting of many PMN in alveoli were also seen in group I (2 animals) and group II (4 animals)(Fig. 5).

Dead animals from group I (3 animals) had intense lung congestion and one of them had pulmonary hemorrhagic zones. Two of those animals had bacterial colonies in tonsils. However, no microscopic evidence explaining the sudden death (such as necrotic foci and/or hemorrhages in other organs) were seen.

3.3. Bacteriology

At necropsy, *Haemophilus parasuis* was isolated from 9 of 10 animals in the PRRSv-*H. parasuis* group (group I) and 9 of 10 animals in group II. Bacteria were isolated from joint, trachea, lung, cerebrospinal fluid (CSF), pericardial and peritoneal fluids (Table 4).

3.4. Virus isolation and serology

Animals were serologically negative to PRRS virus on the day of arrival. All animals challenged with PRRS virus seroconverted by 7–11 days post exposure. Typical viral

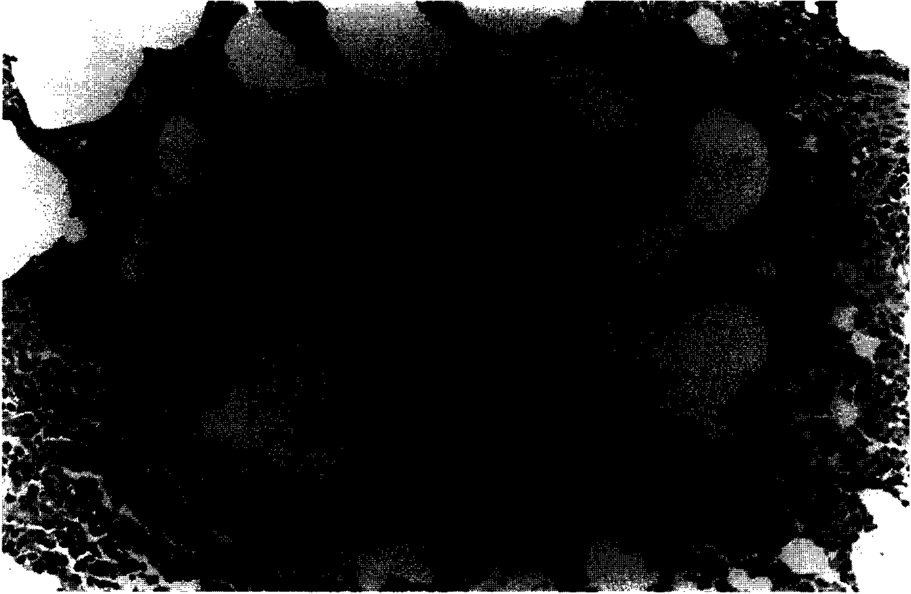

Fig. 4. Marked thickness of alveolar walls (between arrow-heads) in a lung of a pig 10 days post-PRRS virus inoculation (group III), with extensive proliferation of interstitial macrophages. HE 500×.

Fig. 5. Early phases of interstitial pneumonia caused by PRRS virus in a day post-viral inoculation pig (group I). Cellular elements inside alveoli (arrow) corresponding to macrophage proliferation. HE stain 500×.

Table 4
Isolation sites of *H. parasuis* in different challenge groups. (CSF = cerebrospinal fluid, Joint = articular fluid, Perit = peritoneal fluid, Peric = pericardial fluid, Trachea = tracheal swab, Lung = Lung tissue)

Group	CSF	Joint	Perit	Peric	Trachea	Lung
I	4/10	5/10	4/10	3/10	6/10	1/10
II	3/10	3/10	7/10	5/10	5/10	3/10
III	0/5	0/5	0/5	0/5	0/5	0/5
IV	0/5	0/5	0/5	0/5	0/5	0/5

cytopathic effects were seen in 60% of porcine CL2621 cell cultures inoculated with a mixture of tissue samples prepared in HBSS.

4. Discussion

This study is the first experimental report examining a putative interaction between PRRS virus and *Haemophilus parasuis*. Infection with *H. parasuis* alone resulted in more severe clinical signs and lesions, but decreased mortality, compared to animals receiving the dual viral–bacterial challenge. Animals infected only with *H. parasuis* showed a higher prevalence of polyserositis, characterized by pleuritis, pericarditis, polyarthritis, peritonitis and meningitis. In contrast, animals with the combined PRRS virus-*H. parasuis* challenge had only pleuritis and meningitis, or meningitis alone. Catharral–purulent bronchopneumonia was found in six animals from both groups inoculated with the bacteria. Since no other microorganisms were isolated from these lungs, these findings strongly suggest that *H. parasuis* can be associated with pneumonia in appropriately susceptible animals.

A possible explanation of the difference between the group dually infected with the virus–bacterial challenge (group I) and the bacterial challenge alone (group II) in terms of lesions could be the immunomodulating effect of PRRS virus. Previous reports (Molitor et al., 1992; Ohlinger et al., 1992; Galina et al., 1994a) have shown that by 7 days post-infection the virus produces a marked decrease in the percentage and functional ability to release superoxide anion in alveolar macrophages. In contrast, a sharp enhancement of humoral and cell-mediated functions at the systemic level were also found. The fact that group II animals had more severe lesions as compared to animals previously challenged with PRRS virus (group I), may be explained by this enhanced response at the systemic level.

A trend of increased mortality was seen in group I; however, this difference was not statistically significant between group I and II, probably due to limited size. The animals that died had congestive and hemorrhagic pulmonary lesions, with no other pathological lesions recorded. The fact that bacteria were isolated from several different fluids of these animals, together with the rapid occurrence of death may suggest septic shock as the cause of death. It is possible that the local immunosuppresive effects of the virus allowed for rapid bacterial proliferation in some animals that then died.

The fact of observing increased mortality in group I and enhanced polyserositis lesions in group II suggests that the virus has several effects on the pigs simultaneously. Local destruction of phagocytic cells in the lung may lead to a rapid increase of bacterial numbers and death in some animals. In contrast, surviving animals then tend to develop more localized serositis as bacterial lesions because viral infection results in an increased systemic response which may minimize bacterial spread from the lung. Some degree of bacteremia, however, did occur, since bacterial reisolation rates (90%) were the same for groups I and II.

Bacteria were isolated from peritoneal, joint and pericardiac fluids as previously reported (Little, 1970; Morozumi and Nicolet, 1986; Morikoshi et al., 1990). Cerebrospinal fluid was an excellent sample for isolation of the bacteria when the animals show central nervous signs.

In summary, infection of susceptible pigs with PRRS virus followed by *H. parasuis* intratracheal challenge did not result in an increased bacterial polyserositis as compared to the group where *H. parasuis* was the unique challenge. These findings are in contrast to field observations (Vahle et al., 1994; Done and Paton, 1995) where endemic PRRS has been reported to produce an increased occurrence of polyserositis due to *H. parasuis*. Other management factors, such as comingling pigs from different sources, herd health, lack of immunity and other factors precipiting disease should also be considered when interpreting field observations. These results suggest that sudden death may be an important manifestation of this interaction.

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