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## Effect of porcine reproductive and respiratory syndrome virus on subsequent *Pasteurella multocida* challenge in pigs

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### Abstract

This trial was conducted to evaluate the effect of Porcine reproductive and respiratory syndrome virus (PRRSv) on a subsequent challenge with *Pasteurella multocida* in pigs. Sixteen, 3–4 week-old piglets, from a PRRSv and Aujeszky disease virus (ADV) free herd were used. Animals were equally and randomly allocated in four groups which were treated according the following schedule: Group I: negative controls; Group II: inoculation with only PRRSv; Group III: inoculation with PRRSv and *P. multocida*; Group IV: inoculation with ADV and *P. multocida* (positive controls). PRRSv and ADV were inoculated intranasally, at the doses of  $10^{4.6}$  and  $10^{4.5}$  TCID<sub>50</sub>/ml, respectively. Five days later, pigs from groups III and IV were inoculated intranasally, with two ml of a  $10^9$  CFU/ml suspension of equal parts of *P. multocida*, strains A52 and A24. No lesions were observed in piglets of group I. Microscopically, interstitial pneumonia was identified in all piglets of groups II and III and 3/4 piglets from group IV. Bronchopneumonia was detected in 3/4 of the piglets from group III and in all animals of group IV which, additionally, showed meningo-encephalitis and purulent rhinitis. Macroscopically, only piglets of groups III and IV had lung consolidation. However, much lower pneumonic scores (2.3%) were observed in group III, where 3 of 4 piglets were affected. On the other hand, all piglets of group IV showed some degree of pulmonary consolidation, with a mean score of 13.7%. Based on these results, it appears that the role of PRRSv as a initiator of secondary diseases is still undefined, but is probably mild. There was no clear interaction between PRRSv and *Pasteurella multocida* under the conditions and strains tested here. © 1997 Elsevier Science B.V.

**Keywords:** PRRSv; *P. multocida*; Interaction; ADV; Pneumonia

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## 1. Introduction

Porcine reproductive and respiratory syndrome (PRRS) is characterized by reproductive failure (late-term abortions, stillborn, premature farrowings, weak liveborn piglets and mummification) together with respiratory disease in neonates that can last until marketing (Loula, 1991). Recently, PRRS has become an endemic problem, resulting in a variety of clinically apparent secondary disease (Pijoan et al., 1994).

*Pasteurella multocida* is recognized as one of the most important pathogens of swine respiratory diseases (Pijoan, 1992). It is not considered a primary agent of pneumonia, but rather follows infections from other agents. Vaccination against Hog cholera (Pijoan and Ochoa, 1978) as well as infections with either Aujeszky's virus (Fuentes and Pijoan, 1987) and *Mycoplasma hyopneumoniae* (Ciprian et al., 1988) have been shown to predispose pigs to *P. multocida* infection.

PRRS virus (PRRSv) has been shown to interact with *Streptococcus suis* facilitating the production of meningitis by this organism (Galina et al., 1994a). However, its interaction with other respiratory pathogen, such as *P. multocida* has not been reported to date.

The purpose of this study was to evaluate the possible interaction between PRRSV and *P. multocida*, in the production of pneumonia in swine.

## 2. Materials and methods

### 2.1. Animals and housing

Sixteen, 3–4 week old pigs from a PRRSV and Aujeszky disease virus (ADV) negative herd were used. The animals were randomly allocated to four groups of four pigs each and housed in four different isolation rooms.

### 2.2. Microorganisms

PRRSv, strain VR 2332, passage 3 and ADV, strain 4892 (pneumotropic strain) were the viruses utilized. Both viruses were inoculated intranasally. PRRSV was diluted to a dose of  $10^{4.6}$  TCID<sub>50</sub>/ml and ADV to a dose of  $10^{4.5}$  TCID<sub>50</sub>/ml.

Bacteria inocula was prepared to a  $10^9$  CFU/ml suspension with a mixture of equal parts of strains A52 and A24 of *P. multocida* type A, cultured overnight on sheep blood agar. The bacterial challenge was given intranasally.

### 2.3. Experimental design

Group I pigs were used as a negative control. Group II pigs were inoculated only with PRRS virus. Group III pigs were inoculated first with PRRS virus and, five days later, with a 2 ml suspension of *P. multocida*. Group IV pigs received first the ADV and, five days later, *P. multocida*. They were used as a positive control, based on previous reports (Fuentes and Pijoan, 1987) that have shown that ADV inoculation,

Table 1  
Experimental design

Groups	Number of pigs	Day 1	Day 5	Day 10
I (negative control)	4	—	—	necropsy
II (PRRSV)	4	PRRSV	—	necropsy
III (PRRSV + Pm)	4	PRRSV	Pm	necropsy
IV (ADV + Pm)	4	ADV	Pm	necropsy

PRRSV = porcine reproductive and respiratory syndrome's virus inoculation.

ADV = Aujeszky diseases virus inoculation.

Pm = *Pasteurella multocida* inoculation.

followed by *P. multocida*, results in macroscopic pulmonary consolidation. A group of pigs inoculated with *P. multocida* alone was not used since the disease could not be reproduced without the presence of a predisposing agent. Table 1 shows, schematically, the inoculating schedule employed.

Clinical evaluations were performed daily, in all groups. Following the experimental period, all animals were euthanized with an intravenous injection of sodium pentobarbital and necropsy was then performed. Histopathologic studies included samples from lungs, nasal turbinates, trachea, brain, kidneys, liver, spleen, tonsils, lymph nodes, intestines and heart.

Nasal swabs, tracheal swabs and lung samples were collected for microbiological studies. These were done by inoculating samples into brain heart infusion media, supplied with bacitracin (5  $\mu\text{g}/\text{ml}$ ) and lincomycin (5  $\mu\text{g}/\text{ml}$ ). Tracheal and lung samples were also cultured directly onto 5% sheep blood agar. Cultures were incubated at 37°C overnight.

At the beginning and end of the experiment, sera was taken for PRRSV and ADV serology and virus isolation. Tissues were also obtained for virus isolation. PRRSV serology was assessed with an indirect fluorescent antibody test (IFA). ADV serology was conducted using the latex agglutination test (Oren et al., 1993). PRRSV was isolated utilizing porcine alveolar macrophages and the cytopathic effect was measured after 7 days of incubation. ADV isolation was attempted in a porcine kidney cell line.

### 3. Results

All groups showed pyrexia following the viral challenges, which was more pronounced in group IV (Fig. 1). Central nervous clinical signs were detected only in animals with ADV challenge.

Macroscopic pulmonary consolidation was seen, in different proportions, in groups III and IV (Table 2), although group IV, which had ADV challenge had more extensive lesions (2.3% average pneumonic lesions score for group III versus 13.7% for group IV). At the microscopic level, interstitial pneumonia, consisting of a mild mononuclear inflammatory infiltration and thickened alveolar septa, was seen in animals from groups II and III, but also in 3/4 of group IV. Catharralpurulent bronchopneumonia, consisting

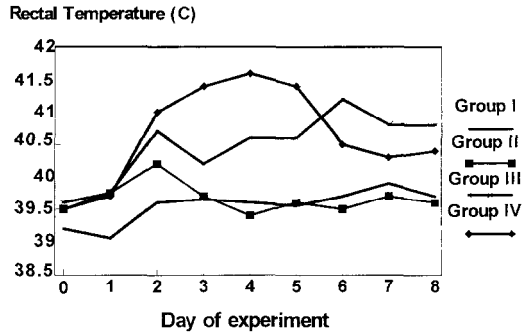


Fig. 1. Rectal temperatures during the experiment. On day 0, animals were challenged with PRRSV or ADV. On day 5, groups III and IV were inoculated with *Pasteurella multocida*.

Table 2  
Lung macroscopic lesions

Group	Pulmonary consolidation	Pneumonic scores (%)				
		pig 1	pig 2	pig 3	pig 4	mean
I	0/4 animals	0.0	0.0	0.0	0.0	0.0
II	0/4 animals	0.0	0.0	0.0	0.0	0.0
III	3/4 animals	1.5	5.7	0.0	2.0	2.3
IV	4/4 animals	1.3	6.8	24.5	22.0	13.7

Table 3  
Histopathological results

Group	Interstitial pneumonia	Broncho-pneumonia	Meningo-encephalitis	Purulent rhinitis
I	0/4	0/4	0/4	0/4
II	4/4	0/4	0/4	0/4
III	4/4	3/4	0/4	0/4
IV	3/4	4/4	4/4	4/4

Table 4  
Results of *Pasteurella multocida* isolation

Group	Trachea	Lung	Total <sup>a</sup>
I	0/4	0/4	0/4
II	0/4	0/4	0/4
III	1/4	1/4	1/4
IV	2/4	3/4	4/4

<sup>a</sup> Total indicates number of animals where the bacteria was isolated.

of large amounts of polymorphonuclear neutrophils in alveoli, was seen in groups III and IV. Meningo-encephalitis and purulent rhinitis were also seen in group IV pigs (Table 3).

In groups III and IV, isolation of *Pasteurella multocida* was successful (Table 4). However, bacteria were only isolated from one of the animals in group III, as compared with group IV where all animals yielded positive cultures.

Additionally, all animals from groups II and III seroconverted to PRRSV. The same was observed with animals from group IV all of them seroconverted to ADV.

#### 4. Discussion

Numerous reports about viral/bacterial interactions in the pig, have been published. However, only a few of these have studied PRRSV and its interaction with other agents. The relationship between PRRSV and *Streptococcus suis* has been proven (Galina et al., 1994b). PRRSV may also interact with other viral agents such as porcine respiratory coronavirus and an unspecified paramyxovirus (Van Rieth et al., 1994). On the other hand, Brun et al., 1994 could not show any difference between groups with PRRSV and Swine influenza virus (SIV) or SIV alone. Unlike other viruses, such as Aujeszky's virus the role of PRRSV as a initiator of secondary disease is still under debate.

In the present experiment, pigs from group III developed pneumonic lesions, but these were very slight when compared with the group where ADV was present. It has been suggested that *P. multocida* alone can cause mild lesions (Hall et al., 1990). The strain A52, when inoculated alone, has previously been shown to cause mild lesions (Fuentes and Pijoan, 1987), similar to those seen here in group III pigs. Therefore, the role of PRRSV virus on the development of pulmonary lesions in group III pigs is unclear. It is probable that the interaction between PRRSV and *P. multocida* did not occur, under the conditions and strains tested here. However, when ADV and the same strains of *P. multocida* were concurrently inoculated, lesions were more severe. These facts cast doubts on the relevance of PRRSV virus, on the development of secondary bacterial bronchopneumonia.

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