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Pathological Studies on Natural and Experimental Porcine Pneumonia Caused by Porcine Reproductive and Respiratory Syndrome (PRRS) Virus

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Abstract

Porcine reproductive and respiratory syndrome (PRRS) was recorded in Chiba Prefecture in 1989. Twenty-four field cases were examined pathologically. The lungs showed consolidations grayish-pink in color in all the cases. Pericarditis was also observed. Histopathologically, the alveolar septa in the lungs were markedly thickened and alveolar spaces were narrow. In the cases with secondary bacterial infection, macrophages and neutrophils infiltrated the alveolar spaces. Lymphoid cells and macrophages also infiltrated the endo-and pericardium, and meninges. A virus was isolated from several field cases and identified as PRRS virus. The hysterectomy-produced colostrums-deprived (HPCD) piglets were inoculated with the virus. In the piglets sacrificed at 7 days after inoculation, interstitial pneumonia which was characterized by a thickening of the alveolar septa was observed. Severe lesions were observed at 28 days after inoculation, and they subsided 42 days after inoculation. The lesions in the piglets in which PRRS virus and Mycoplasma hyorhinis were inoculated, were more severe than those in the piglets in which only PRRS virus was inoculated. As a result of these observations, it was considered that Mycoplasma hyorhinis increased the severity of the lesions in the piglets infected with PRRS virus.

Discipline: Veterinary pathology
Additional key words: arterivirus, mycoplasma

Introduction

Porcine reproductive and respiratory syndrome (PRRS) was first recognized in 1987 in the U.S.A.[2,3]. A similar syndrome was reported in Canada, Germany, the Netherlands, England, Belgium, and Spain. PRRS virus was first isolated in Lelystad, Netherlands[8] in 1991. PRRS virus infection caused abortion in sow[6]. Young pigs suffering from this disease showed pronounced hyperpnea, fever and interstitial pneumonia[2,3]. This disease was popularly called Heko-Heko disease in Japan. Heko-Heko disease has been recognized in Chiba Prefecture, Japan, since October 1989[7]. It occurred in large farms under poor sanitary conditions and 10% of the piglets died, although infertility and abortion were not observed. We examined these cases pathologically, virologically and bacteriologically.

A virus isolated from Heko-Heko disease was designated as Chiba 92-1[5] and this virus was identified as PRRS virus. We inoculated this virus into hysterectomy-produced colostrum-deprived (HPCD) piglets in order to reproduce the disease. We describe the results in this report.

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Materials and methods

Field cases: Twenty-four piglets about 2 month-old which showed symptoms of Heko-Heko disease were sent to the National Institute of Animal Health from 2 farms in Chiba Prefecture for examination.

Bacteriological examinations: The general bacteriological examinations were performed using blood agar plates. As for the isolation of *Haemophilus* spp., a special medium was used. Mycoplasma was isolated using mucin PPLO broth (M-broth).

Virological examinations: Sera and emulsion samples prepared from the lungs, liver, spleen and mesenteric lymph nodes were inoculated into the porcine kidney cell line cultures or alveolar macrophage cultures from HPCD pigs.

Experimental inoculation: HPCD piglets were maintained in stainless tubes covered with a plastic film. The piglets were fed recommended amounts of commercial milk substitute 2 or 3 times a day.

Four piglets were inoculated with 10^5 Chiba 92-1 strain of PRRS virus intranasally at 5 days of age. These piglets were euthanatized at 7, 14, 28, and 42 days postinoculation (pi.).

Two piglets were inoculated with *Mycoplasma hyorhinis* (*Mhr*) at 5 days after inoculation with PRRS virus at 21 days of age. One was moribund at 10 days after inoculation with PRRS virus. The other was euthanatized at 26 days after inoculation with PRRS virus.

Two 26-day-old piglets were inoculated with *Mhr*. One of them was euthanatized for examinations at 21 days pi. and the other piglet was not necropsied because it did not show clinical signs.

Histopathological examinations: At necropsy, the organs from field and experimental cases were fixed in 10% phosphate-buffered formalin. These organs were embedded in paraffin, sectioned at 5 μm and stained with hematoxylin and eosin (HE).

Immunohistochemistry: The *Mhr* antigen in the lung specimens from 6 field cases and one experimental case, which was inoculated with both PRRS virus and *Mhr*, was examined using the avidin biotin complex method kit (Vectastain Ca., U.S.A.).

Results

Field cases: The lungs from 24 field cases showed consolidations grayish-pink in color, and sometimes fibrinous pleuritis. Pericarditis was found in 12 cases and peritonitis in one case.

Microscopically, interstitial pneumonia characterized by a thickening of the alveolar septa was observed in 20 cases (Plate 1a). The thickening of the alveolar septa was mainly due to the proliferation of alveolar epithelial cells and infiltration of macrophages (Plate 1b). The macrophages infiltrated the alveolar spaces in all cases, and neutrophils coexisted in 12 cases. The cell infiltrations were pronounced in the case of bacterial infection. In 14 cases, there were perivascular and peribronchiolar infiltrations of plasma cells. Fibrinous pleuritis was seen in 5 cases. Macrophages and a few neutrophils infiltrated the pleura in these cases.

The *Mhr* antigen was detected on the surface of the bronchiolar epithelial cells in 5 out of 6 cases examined.

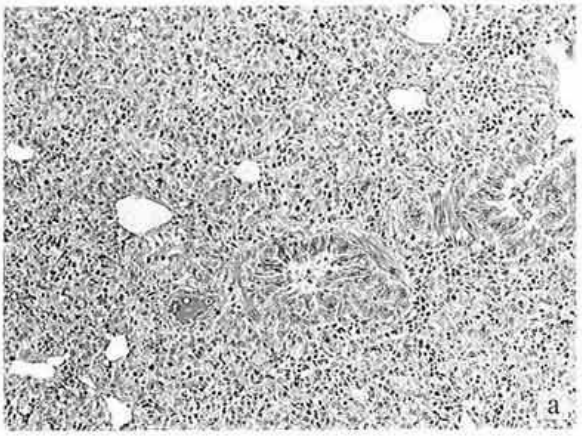

Plate 1a. Marked thickening of alveolar septa in lung (field case)
Hematoxylin and eosin (HE) stain. (× 100)

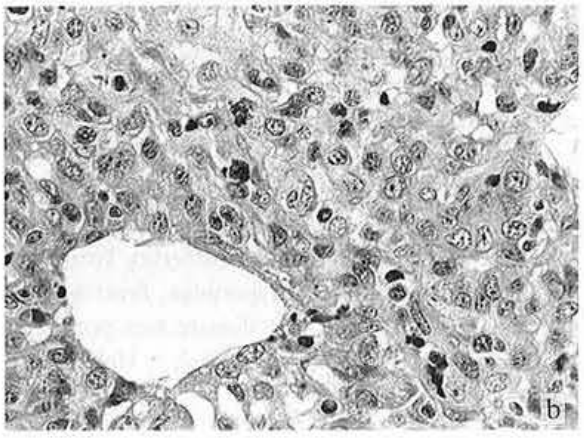

Plate 1b. High magnification of Plate 1a
Proliferation of alveolar epithelial cells and macrophages. HE stain. (× 400)

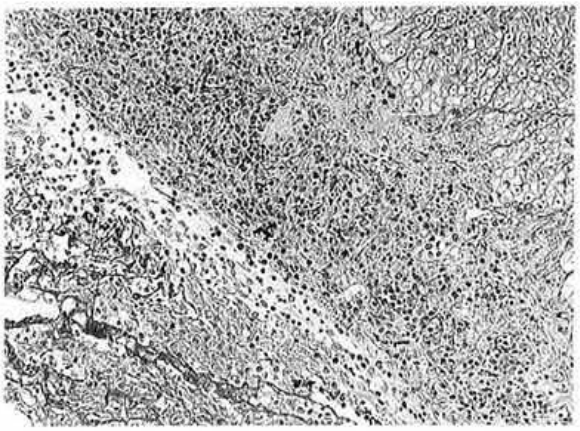

Plate 2. Heart in field case
Macrophages and lymphocytes infiltrate the pericardium. There are fibrin deposits around the pericardium. HE stain. (× 100)

Macrophage and lymphocyte infiltrations were seen in the pericardium, endocardium and occasionally in the myocardium (Plate 2). Mild nonpurulent meningitis was observed in 14 cases, perivascular cuffing in 5 cases and accumulation of glial cells in 5 cases. In the other major organs there were no microscopical lesions.

Bacteriologically, in most of the field cases examined, 10^6–10^8 cfu/g of *Mhr* were isolated. *Haemophilus parasuis, Actinobacillus pleuropneumoniae* and *Pasteurella multocida* were occasionally isolated.

Virologically, when porcine kidney cell lines were used, reovirus and coronavirus were isolated from the lungs of 2 cases. Chlamydia were isolated from the mesenteric lymph nodes from 4 out of 6 cases examined. PRRS virus was isolated from the sera and lungs of 5 out of 6 cases examined[5].

Experimental cases: The piglets which were inoculated only with PRRS virus or *Mhr* did not exhibit gross lesions. Fibrinous pleuritis was observed in the piglet inoculated with PRRS virus and *Mhr*.

Microscopically, interstitial pneumonia was characteristic in the lungs of the piglets inoculated with PRRS virus.

In a piglet which was euthanatized at 7 days pi., interstitial pneumonia was sparsely distributed, but it was not severe (Plate 3). Alveolar septa were thickened due to the infiltration of macrophages and lymphocytes. Small accumulations of degenerated cells with pyknotic nuclei were present in the alveolar septa.

In a piglet euthanatized at 14 days pi., the lesions of interstitial pneumonia became more severe and spread over larger areas of the lungs (Plate 4). The marked thickening of the alveolar septa was due to macrophage and lymphocyte infiltrations.

The piglet which was euthanatized at 28 days pi., showed the most severe lung lesions. The alveolar septa were thickened by proliferation of alveolar epithelial cells. Plasma cell infiltration was seen around the peribronchiolar and perivascular areas in the lungs. Macrophages and lymphocytes infiltrated the pericardium and endocardium. Mild meningitis was also seen.

Although the lesions in a piglet at 42 days pi. were milder, the cell components in the lesions were

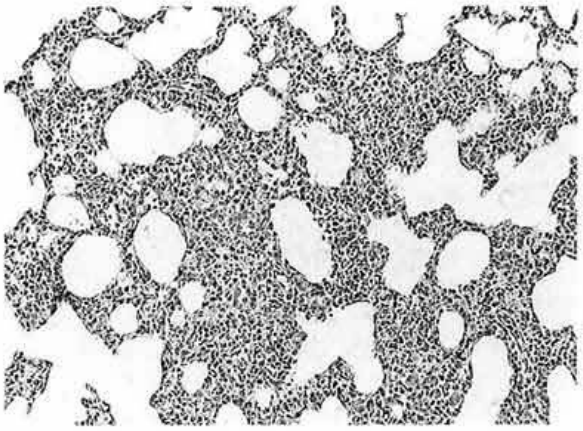

Plate 3. Lung of piglet at 7 days after inoculation of porcine reproductive and respiratory syndrome (PRRS) virus
Interstitial pneumonia is not severe and alveolar septa are thickened by infiltration of macrophages, lymphocytes and degenerated cells. HE stain. (× 100)

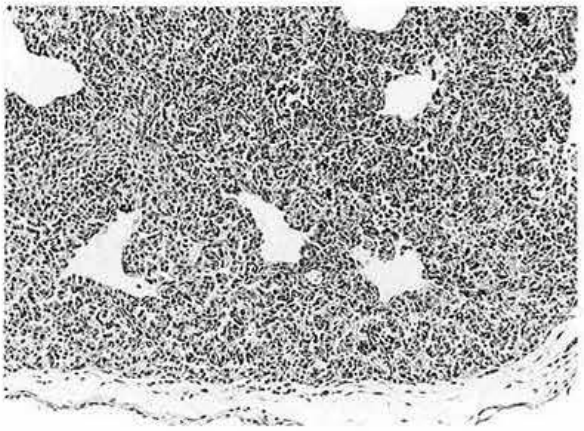

Plate 4. Lung of piglet at 14 days after inoculation of PRRS virus
Alveolar septa are markedly thickened by infiltration of macrophages and lymphocytes. HE stain. (× 100)

the same as those in the piglet at 28 days pi.

One of two piglets which were inoculated with both PRRS virus and *Mhr* was moribund at 10 days pi. of PRRS virus, and showed the most severe lung lesions among the experimental cases. Alveolar epithelial cells proliferated and macrophages infiltrated the alveolar septa and alveolar spaces (Plate 5). Cellular debris accumulated in the alveolar septa. *Mhr* antigen was detected on the surface of the bronchiole by immunohistochemistry. The other piglet which was euthanatized at 26 days after inoculation with PRRS virus, exhibited milder lesions with the same cell components as in the former case. Plasma cells infiltrated the peribronchiolar areas in the lungs. Pleuritis, pericarditis and endocarditis (Plate 6) as well as meningitis (Plate 7) were also observed (Table 1).

In the cases which were inoculated with *Mhr* alone, there were no recognizable lesions.

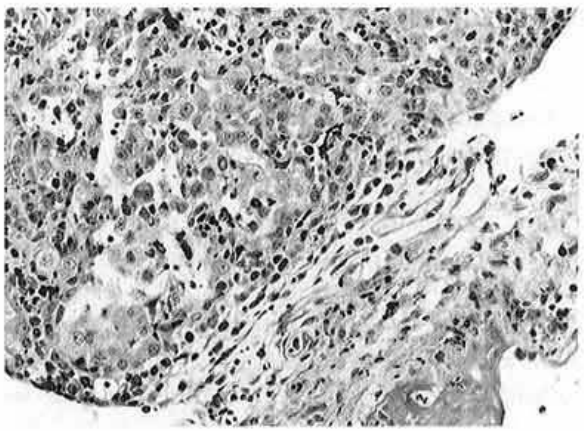

Plate 5. Lung of piglet at 10 days after inoculation of PRRS virus and 5 days after inoculation with *Mycoplasma hyorhinis* (*Mhr*)
Alveolar septa are markedly thickened by the proliferation of alveolar epithelium and infiltration of macrophages. Fibrin deposits in pleura. HE stain. (× 100)

Discussion

It was reported that PRRS is characterized by interstitial pneumonia in young pigs[1] and abortion in sows[6]. In the herd in which Heko-Heko disease occurred, neither abortion nor reproductive disorders were observed by farmers. The major clinical signs consisted of laborious abdominal respiration in the piglets[7]. The PRRS virus was isolated from the lungs and sera of the diseased piglets and it was designated as Chiba 92-1[5]. Since the inoculation of this virus induced interstitial pneumonia, as in the field cases, it was assumed that PRRS virus was the causal agent of Heko-Heko disease.

Histologically, interstitial pneumonia was characterized by the thickening of the alveolar septa and plasma cell infiltration in perivascular and peribronchiolar areas. These lesions were commonly observed in the field cases. In experimental cases, slight interstitial pneumonia was already observed at 7 days pi. Up to 14 days pi. the thickening of the alveolar septa was mainly due to the infiltration of macrophages. Thereafter, macrophages in the alveolar septa were gradually replaced by a proliferation of alveolar epithelial cells. The lung lesions showed the most severe appearance at 28 days pi. and the lesions at 42 days pi. became milder in appearance. Therefore,

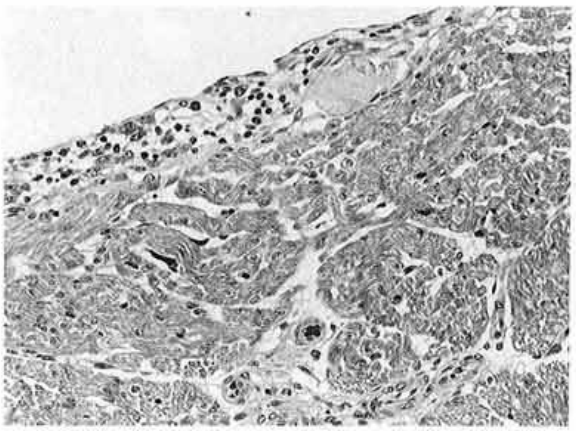

Plate 6. Heart of the same case as in Plate 5
Plasma cells and macrophages infiltrate the endocardium. HE stain. (× 200)

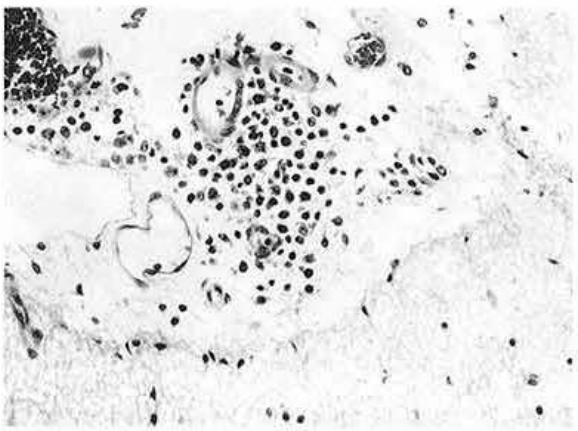

Plate 7. Brain of piglet at 26 days after inoculation with PRRS virus and 21 days after inoculation with *Mhr*
Lymphocytes and macrophages aggregate in the meninges. HE stain. (× 200)

Table 1. Pathological lesions in field cases and experimental cases

		Field cases PRRSV[a]				Experimental cases PRRSV + *Mhr*[b]	
		7	14	21	42 d[c]	10	26 d[d]
Alveolar septa							
Thickening	20[e]/24[f]	+	+	+	+	+	+
Cellular debris	0/24	+	+	−	−	+	+
Alveolar space							
Macrophages	24/24	−	+	−	−	+	+
Neutrophils	12/12	−	+	−	−	−	−
Peribronchiole							
Plasma cells	14/24	−	−	+	+	+	+
Pleuritis	5/24	−	−	−	−	+	+
Heart							
Carditis	14/24	−	−	+	+	+	+
Brain							
Meningitis	14/24	−	−	+	+	+	+
Cuffing	5/24	−	−	+	−	+	+
Glial nodules	5/24	−	−	+	−	−	−

a): Porcine reproductive and respiratory syndrome virus, b): *Mycoplasma hyorhinis*, c): Days after inoculation, d): Days after inoculation of PRRS virus (*Mhr* was inoculated at 5 days after PRRS virus inoculation.), e): Number of positive pigs, f): Number of pigs examined.
+: Lesions are present, −: Lesions are absent.

it appears that interstitial pneumonia caused only by PRRS virus could be cured naturally, if there is no secondary infection with mycoplasma or bacteria.

Mild nonpurulent meningitis and peri- and endocarditis were often observed in the field cases. These lesions were reported by other authors[1,4,9] and our experiments also confirmed the same findings. It was considered that the PRRS virus had an affinity not only to the lung tissues but also to the heart tissues and meninges.

Mhr was isolated from the lungs in most of the field cases. However, the piglets inoculated only with *Mhr* did not exhibit any lesions in the lungs. On the other hand, the pigs inoculated with both PRRS virus and *Mhr* showed more severe lesions than those inoculated with PRRS virus alone. These results suggest that *Mhr* was not the causal agent of interstitial pneumonia. However, *Mhr* may produce severe lesions in the piglets which have been infected with PRRS virus.

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