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Pathological Investigations on Bovine Pheumonic Pasteurellosis by Use of Immunoperoxidase Technique

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Abstract
Lesions of calf pneumonia caused by *Pasteurella haemolytica* or *P. multocida* were examined histopathologically using an immunoperoxidase technique for the detection of the bacterial antigen. The results indicated that: 1) multifocal coagulation necrosis sharply demarcated by degenerated leukocytes was a characteristic pulmonary lesion caused by *P. haemolytica*, 2) other bacteria than *P. haemolytica* sometimes caused necrotic lesions in lungs of calves with naturally acquired pneumonia, 3) the lack of multifocal coagulation necrosis in P. multocida pneumonia was the major difference from *P. haemolytica* pneumonia, and 4) the immunoperoxidase technique can be used for the accurate diagnosis of pneumonia in cattle.

Discipline: Animal health
Additional key words: pneumonia, Pasteurella haemolytica, P. multocida

Introduction

Pneumonic pasteurellosis is a common disease of cattle. *P. haemolytica* and *P. multocida* are the causal agents of the disease. Annual loss attributed to the disease in the United States has been estimated at 25 million dollars, excluding the cost for treatment and preventive measures[10]. In Japan many calves in mass-rearing facilities also die of pneumonic pasteurellosis[7].

Although many investigators have reported on the occurrence and bacteriological examination of calf pneumonia, there are few detailed reports on the histopathological changes of this disease. The purpose of the present studies was to analyze the histological changes associated with the disease caused by *P. haemolytica* or *P. multocida* along with the distribution of the bacterial antigen in the lung lesions using the avidin-biotin-complex immunoperoxidase technique (ABCIT).

Pneumonic lesions induced by inoculation of *P. haemolytica*[3]

The lungs of 6 calves were inoculated with *P. haemolytica* (serovar 1) using an intra-tracheal catheter. The lungs were examined histologically. Avidin-biotin-complex immunoperoxidase technique (ABCIT) was employed for the detection of *P. haemolytica* (serovar 1) antigen in tissues fixed in formalin and embedded in paraffin.

In 5 out of the 6 calves, numerous nodules, up to 1 cm in diameter, were observed in the lungs (Plate 1). The nodules were hard, slightly raised, and red to tan in color. Histologically, the nodules consisted of coagulation necrosis surrounded by a dense zone of infiltrating leukocytes (Plate 3). *P. haemolytica* antigen was detected diffusely in the area of coagulation necrosis (Plate 4).

Pneumonic lesions induced by inoculation of *P. multocida* in calves[4]

Six calves were inoculated with *P. multocida* (serovar D) using an intra-tracheal catheter. The lungs were examined histologically and immunohistologically using the ABCIT for the detection of the bacterial antigen of inoculated *Pasteurella*.

Lobar pneumonic lesions with severe pleuritis were observed in 4 out of the 6 calves. The lesions consisted of congestion and consolidation with a homogeneous dark red color on the visceral and cut surfaces (Plate 2). Histologically, the lesions consisted of fulminating bronchopneumonia and fibrinopurulent pleuritis. Alveoli and bronchioles

Plate 1.

Plate 2.

Plate 3.

Plate 4.

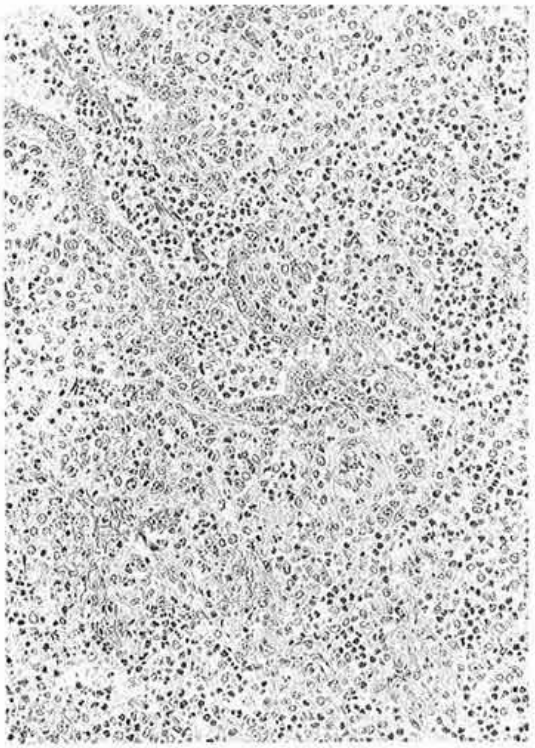

Plate 5.

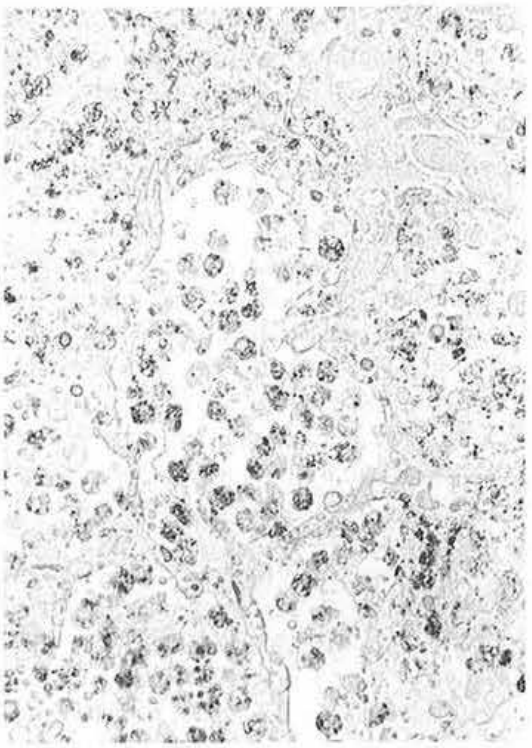

Plate 6.

Plate 1. Parietal surface of posterior cranial lobe of left lung in a calf experimentally infected with *Pasteurella haemolytica*
Pneumonic lesions consist of congestion, consolidation and numerous nodules up to 1cm in diameter.

Plate 2. Parietal surface of posterior cranial lobe of left lung in a calf experimentally infected with *P. multocida*
Pneumonic lesions consist of homogeneous congestion and consolidation.

Plate 3. Photomicrograph of an area of coagulation necrosis in the lungs of a calf experimentally infected with *P. haemolytica*
Necrotic tissues are surrounded by a dense zone of leukocytes.
(Hematoxylin and Eosin (H & E) stain, × 6).

Plate 4. Photomicrograph of the same specimen as in Plate 3, stained by the use of the avidin-biotin-complex immunoperoxidase technique (ABCIT)
Necrotic tissues display a specific reaction against *P. haemolytica* antiserum.
(× 6).

Plate 5. Photomicrograph of the lung from a calf experimentally infected with *P. multocida*
Alveoli and bronchioles are filled with many neutrophils and macrophages.
(H & E stain, × 250).

Plate 6. Photomicrograph of the same specimen as in Plate 5, stained by use of ABCIT
P. multocida antigen is found in the cytoplasm of neutrophils and macrophages.
(× 400).

were filled with neutrophils, macrophages and a small amount of fibrin (Plate 5). *P. multocida* antigen was associated with these lesions. In the alveoli, bronchioles and bronchi, the bacterial antigen was detected in the cytoplasm of infiltrating neutrophils and macrophages (Plate 6).

Pneumonic lesions in calves naturally infected with *P. haemolytica*[5)]

In a total of 194 calves with pneumonia, pathological and bacteriological examinations were carried out. *P. haemolytica* was isolated from 42 out of the 194 calves. Lung tissues of these 42 cases were examined using the ABCIT for the detection of *P. haemolytica* antigen. In addition, 23 cases were selected from 152 cases from which *P. haemolytica* was not isolated, and also used for the present study.

The results are shown in Table 1. Multifocal necrosis was observed in the lungs of 25 out of 42 calves (59.5%) and the *P. haemolytica* antigen was detected in 22 out of the 25 calves (88.0%). The calves were divided into 3 groups according to the number of *P. haemolytica* isolated. Calves from which *P. haemolytica* was not isolated were classified into the fourth group. The rate of bacterial antigen detected by the ABCIT was 66.6% (28/42) on the average, reaching a level of up to 85.7% (18/21) in the group from which the largest number of *P. haemolytica* was isolated. In addition, coagulation necrosis was also observed in 7 out of 23 calves from which *P. haemolytica* was not isolated, and the *P. haemolytica* antigen was detected in 5 out of the 7 calves.

Relationship between necrotic lesions and causative bacteria in lungs of calves with naturally acquired pneumonia[6)]

The lungs of 1,247 calves were collected from a slaughterhouse. Of these, 53 pairs of the lungs exhibiting necrotic lesions were used for this study. The lungs were examined histologically and ABCIT was used for the detection of bacterial antigens including *P. haemolytica, P. multocida, Haemophilus somnus, Streptococcus* sp., *Staphylococcus* sp. and *Actinomyces pyogenes*.

The results are shown in Table 2. Four types of necrotic lesions were identified on the basis of morphological examination as follows: Type 1 lesions

Table 1. Relationship among *Pasteurella haemolytica* (P.h.) isolation, focal necrosis and P.h. antigen in lungs of calves naturally infected with P.h.

Group	No. of isolated P.h. colonies[a)]	Total no. of calves	Relationship between lesions and antigen		
			Focal necrosis[b)]	P.h. antigen[c)]	No. of calves
1	+++	21	+	+++	14
			+	−	1
			−	+++	4
			−	−	2
2	++	6	+	+++	3
			+	+	1
			+	−	1
			−	−	1
3	+	15	+	+++	4
			+	−	1
			−	+++	1
			−	++	1
			−	−	8
4	−	23	+	+++	3
			+	++	1
			+	+	1
			+	−	2
			−	−	16

a): − ; no growth, + ; <9 colonies/plate, ++ ; 10–49 colonies/plate, +++ ; >50 colonies/plate.
b): + ; present, − ; absent.
c): − ; not detected, + ; weak and partial reaction, ++ ; moderate reaction, +++ ; strong and diffuse reaction.

Table 2. Relationship between microscopic findings of necrotic lesions and presence of bacterial antigen in lungs of calves with naturally acquired pneumonia

Lesion type	Total no. of calves	Microscopie findings of necrotic lesions	No. of calves	Bacterial antigen[a)]
1	16	Coagulation necrosis surrounded by a dense zone of numerous degenerated leukocytes	9	P.h.
			3	H.s.
			1	Str.
			1	Str. and A.p.
			1	Sta.
			1	-
2	22	Although the lesions were similar to those of type 1, the central area of the lesions was severely affected, the alveolar architecture was lost and the area was surrounded by a thin layer of degenerated leukocytes.	2	P.h.
			1	P.h. and H.s.
			2	P.h. and A.p.
			3	H.s.
			6	A.p.
			1	A.p. and Str.
			4	Str.
			3	-
3	8	Swirling accumulation of degenerated leukocytes	1	P.h.
			1	H.s.
			1	Sta.
			1	Sta. and A.p.
			4	-
4	7	Round abscess-like lesions demarcated by a zone with a large number of degenerated neutrophils	2	Str.
			1	Str. and A.p.
			2	A.p.
			1	Sta.
			1	-

a): P.h.; *Pasteurella haemolytica*, H.s.; *Haemophilus somnus*, Str.; *Streptococcus* sp., Sta.; *Staphylococcus* sp., A.p.; *Actinomyces pyogenes*.

consisted of coagulation necrosis surrounded by a dense zone of numerous degenerated leukocytes; Type 2 lesions were similar to the lesions of type 1, but the central area of the lesions was markedly necrotic resulting in the loss of alveolar architecture and surrounded by a thin layer of degenerated leukocytes; Type 3 lesions showed a small swirling accumulation of degenerated leukocytes; Type 4 lesions consisted of necropurulent areas resembling abscesses. By the use of the ABCIT, *P. haemolytica* antigen was confirmed to be associated with the necrotic areas in 9 out of 16 cases with type 1 lesions and in some cases of type 2 and type 3 lesions. In 3 cases of type 1 lesions, *H. somnus* antigen was detected in the necrotic areas.

Discussion

Although generally pneumonic lesions caused by *P. haemolytica* consist of fulminating fibrinous lobar pneumonia[10], coagulation necrosis sharply demarcated by degenerated leukocytes is considered to be a characteristic lesion[1]. However, the relationship between the coagulation necrosis and distribution of the bacterial antigen has not been clarified. Detection of *P. haemolytica* antigen has been reported, in frozen sections, using direct or indirect fluorescent antibody technique[8,9]. In this method, it was difficult to observe the relationship between the histological structure and distribution of the bacterial antigen due to observation under a dark-field microscope. In the present study, the bacterial antigen was demonstrated in the coagulation necrosis lesions using the ABCIT. This finding confirmed that coagulation necrosis was an important pulmonary lesion in both experimental and natural *P. haemolytica* pneumonia.

To determine whether other bacteria produced similar lesions as those caused by *P. haemolytica*, therefore we examined the relationship between the necrotic lesions and causative bacteria in lungs of calves with naturally acquired pneumonia. The results indicated that *H. somnus* was occasionally associated with the necrotic lesions similar to those of *P.*

haemolytica pneumonia[2].

P. multocida tends to cause a fibrinopurulent bronchopneumonia[1]. Although a few investigators have reported on the histological changes, the descriptions were not detailed. Therefore we examined the relationship between the histological lesions and the distribution of the bacterial antigen in *P. multocida* pneumonia in order to differentiate *P. multocida* pneumonia from *P. haemolytica* pneumonia. Histological findings of the infected calves indicated that the pulmonary lesions caused by *P. multocida* consisted essentially of bronchopneumonia and the lack of focal necrosis was the most striking difference from *P. haemolytica* pneumonia.

We used the ABCIT for the detection of the bacterial antigen. *P. haemolytica* antigen was detected not only in the coagulation necrosis from which the bacteria were isolated but also in the lesions from which the bacteria were not isolated[5]. The ABCIT may thus enable to detect live and dead solubilized bacterial antigen. The technique can be used for the accurate diagnosis of pneumonia in cattle.

Conclusion

Coagulation necrosis was confirmed to be a characteristic lesion of pneumonia caused by *P. haemolytica*. Calves infected with *P. haemolytica* do not recover due to the formation of coagulation necrosis in the pneumonic lesions. In the future, studies on the pathogenesis and cause of the necrosis should be carried out.

The present results suggested that the immunoperoxidase technique could be used for the diagnosis of calf pneumonia. This method is reliable, inexpensive and fairly rapid compared with bacterial isolation, and allows retrospective studies.

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