Immunoperoxidase Evaluation of Pneumonic Lesions in Calves Naturally Infected with *Pasteurella haemolytica*

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**ABSTRACT.** Immunoperoxidase technique was applied for pathological study on naturally occurring pneumonic tissues of calves from which *Pasteurella haemolytica* was isolated. Multifocal necrosis occurred in the lungs of 25 out of 42 calves (59.5%) and *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. The positive rate of the bacterial antigen detected by the technique was 66.6% (28/42) on the average, reaching up to 85.7% (18/21) in the group from which the largest number of *P. haemolytica* was isolated.—*KEY WORDS:* calf, immunoperoxidase technique, *Pasteurella haemolytica*, pneumonia.

Pneumonia caused by *Pasteurella haemolytica* is an important disease in calves and diagnosed by the bacterial isolation [5, 6]. In our previous studies on calves experimentally infected with *P. haemolytica* [2], multifocal coagulation necrosis was considered as a characteristic pulmonary lesion and occurred closely related with *P. haemolytica* antigen.

The purpose of the present study is to confirm the correlation between the necrotic lesion and bacterial antigen in calves naturally affected with pneumonic pasteurellosis, referring to bacterial isolation results.

**MATERIALS AND METHODS**

*Animals:* A total of 194 pneumonic calves were examined pathologically and bacteriologically. They were 10 to 191 days of age, and dead or slaughtered due to unfavourable prognosis in August 1973 to February 1977 in three mass rearing facilities for male dairy calves in Aomori prefecture [3]. *P. haemolytica* was isolated from 42 out of the 194 calves and did not from 23 calves, and both the isolated and non-isolated cases were used for the present study.

*Bacterial isolation:* Six tissue blocks from the lungs of each calf were collected and cultured on sheep blood nutrient agar, MacConkey, BTB, and chocolate nutrient agar plates and incubated at 37°C for 24–48 hr [3]. These blocks included grossly pneumatic or normal areas. The bacteria isolated were identified by the methods reported elsewhere [1].

*Histological examination:* Lung tissues were fixed in buffered 10% formalin, and paraffin sections stained with hematoxylin and eosin (H-E).

*Immunoperoxidase technique:* ABC immunoperoxidase technique (ABCIT) was done according to the method previously described [2].

**RESULTS**

*Bacterial isolation:* The 42 calves were divided into three groups according to the
number of _P. haemolytica_ isolated (Table 1). Calves from which _P. haemolytica_ was not isolated were classified into the fourth group. A large number of _Pasteurella multocida_ were also isolated from many cases of groups 2–4.

**Histopathological and Immunoperoxidase findings:** Table 1 shows the relationship among necrosis, isolated _P. haemolytica_ and the bacterial antigen in the lungs.

In groups 1 and 2, multifocal necrosis was observed in 20 out of 27 calves (74%) (Fig. 1A). In the center of the lesions, all the alveoli were filled with fibrin, serous exudate and some degenerating cells, sometimes with hemorrhages. In the margin of the necrotic areas there were a large number of infiltrating cells showing degenerating nuclei (Fig. 2). Alveoli around the necrotic lesions were packed with fibrin, macrophages and some neutrophils. _P. haemolytica_ antigen was recognized in necrotic alveolar walls, fibrinous and serous exudate, and degenerated leukocytes in the center of the necrotic lesions by the ABCIT in 18 out of the 20 calves (Fig. 1B). The antigen was also found in the cytoplasm of degenerated leukocytes in the margin of the necrotic areas and macrophages in the alveoli around them. In each one case of groups 1 and 2, the necrotic areas were demarcated by a thin margin composed of a small number of degenerated cells, but the antigen was detected in the same pattern as in other cases.

Seven calves in groups 1 and 2 had no necrotic lesions, however, 5 out of them had focal lesions consisting of degenerated cells, macrophage and some neutrophil exudation into alveoli as observed around the necrotic areas described above. The bacterial antigen was demonstrated in the exudates in 3

<table>
<thead>
<tr>
<th>Group</th>
<th>Isolated No. of P.h. colonies</th>
<th>Total No. of calves</th>
<th>Relationship between lesion and antigen</th>
<th>P.h. antigen&lt;sup&gt;c&lt;/sup&gt;</th>
<th>No. of calves</th>
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<td>1</td>
<td>++</td>
<td>21</td>
<td>+ &lt;br&gt;++ &lt;br&gt;− &lt;br&gt;− &lt;br&gt;− &lt;br&gt;− &lt;br&gt;−</td>
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<td>2</td>
<td>+</td>
<td>6</td>
<td>+ &lt;br&gt;++ &lt;br&gt;+ &lt;br&gt;− &lt;br&gt;− &lt;br&gt;− &lt;br&gt;−</td>
<td>3 &lt;br&gt;1 &lt;br&gt;1 &lt;br&gt;1 &lt;br&gt;8 &lt;br&gt;8</td>
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<td>3</td>
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<td>4 &lt;br&gt;1 &lt;br&gt;1 &lt;br&gt;1 &lt;br&gt;8</td>
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<td>4</td>
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<td>23</td>
<td>+ &lt;br&gt;++ &lt;br&gt;+ &lt;br&gt;− &lt;br&gt;− &lt;br&gt;− &lt;br&gt;−</td>
<td>3 &lt;br&gt;1 &lt;br&gt;1 &lt;br&gt;2</td>
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<sup>a</sup>: no growth, <sup+b</sup>: <9 colonies/plate, <sup++</sup>: 10-49 colonies/plate, <sup+++</sup>: >50 colonies/plate.
<sup>b</sup>: +: existed, −: not existed.
<sup>c</sup>: −: not detected, +: weak and partial, ++: moderate, +++: strong and diffuse.
out of 4 calves examined in group 1. In the remaining 2 calves of group 1, pulmonary lesions consisted of diffuse infiltration of macrophages and neutrophils and fibrinous exudation in the alveoli and bronchi. The bacterial antigen was detected in the cytoplasm of many macrophages in one of the calves.

In group 3, necrosis was detected in 5 out of 15 calves (33.3%). The morphological features were similar to those of groups 1 and 2. *P. haemolytica* antigen was demonstrated in 4 out of the 5 calves and in another 2 cases. One had severe haemorrhagic pneumonia and the other had moderate bronchopneumonia. The bacterial antigen was found in the cytoplasm of some macrophages and neutrophils in the latter, from which a large number of *P. multocida* were isolated.

In group 4, 7 calves had necrotic areas similar to those observed in other groups. Five of them had the bacterial antigen, but no *P. haemolytica* was isolated in all 7 calves.

**DISCUSSION**

In a previous report [2], necrosis was one of the most characteristic lesions of *P. haemolytica* pneumonia which was experimentally induced. In the present paper, pulmonary necrosis was recognized in 25 out of 42 calves (59.5%) from which *P. haemolytica* was isolated. Moreover, *P. haemolytica* antigen was demonstrated in 22 out of the 25 calves (88.0%). Therefore, multifocal necrosis might be characteristic in both natural and experimental *P. haemolytica* pneumonia.

The histological features and bacterial antigenic distribution pattern in the lung necrosis of the present animals resembled those of the experimental case [2]. Howev-
er, necrotic areas in a few cases were somewhat different and had the marginal zone of the fewer degenerated cells, which were considered to be due to a different stage of infection.

Recently, immunoperoxidase technique on paraffin embedded sections has been employed for many infectious diseases of domestic animals. In the present report, we compared the results of bacterial isolation with those of the ABCIT for detection of the bacterial antigen. The positive rate of the bacterial antigen detected by the ABCIT paralleled with the number of *P. haemolytica* isolated; the positive rate was 85.7% in group 1, 66.6% in group 2, and 40.0% in group 3, respectively. The same positive data using the ABCIT were reported in the spleen of mice experimentally infected with *Brucella abortus* and in the placenta of spontaneous bovine and caprine brucellosis [4]. Thus, it was suggested that the positive results of bacterial isolation without specific reaction to ABCIT in tissues were due probably to a low concentration in the lesions or focal accumulation of bacteria outside of them. Moreover, immunoperoxidase technique seems to detect vivid and dead solubilized bacterial antigens.

In conclusion, the present results suggest that the immunoperoxidase technique can be available for the diagnosis of *P. haemolytica* pneumonia. This method is reliable, inexpensive and fairly quick compared with bacterial isolation, and allows retrospective studies.

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REFERENCES

要約

野外の子牛における Pasteurella haemolytica による肺炎病変の免疫組織学的研究：播谷 壽・石野清之・岡基・中沢宗生・小林 勝・成田 寛・渋沢隆安（農林水産省家畜衛生試験場）——Pasteurella haemolytica (P. h.) が分離された野外例子牛肺炎組織について、酵素抗体法を応用して病理学的に検査した。その結果、多発性壊死巣が42頭のうち25頭（59.5%）に観察され、それらのうち22頭の壊死巣に P. h. 抗原が検出された。子牛は P. h. の分離菌量によって3群に分けられたが、酵素抗体法による菌抗原の検出率は平均で66.6%、分離菌量が多い群では85.7%に達した。