Pathological Studies on Bovine Pneumonia in Special Reference to Isolation of Mycoplasmas

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(Received for publication October 3, 1975)

Abstract. Pathological examination was made on 48 cases of pneumonic lungs in cattle and calves, which had been collected in Miyagi and Tochigi Prefectures between April 1973 and March 1974. From those cases T-mycoplasmas were isolated at a high incidence, particularly from 90% of cases under 6 months of age. Pneumonic lesions were characterized by bronchitis or bronchiolitis, catarrhal bronchopneumonia, supplicative bronchopneumonia, or others including fibrous and interstitial pneumonia and atelectasis. Severe supplicative bronchopneumonia was seen in many calves under 6 months of age, while mild bronchitis or bronchiolitis and catarrhal bronchopneumonia were predominant in many cases 7 to 24 months of age. In many cases having only mycoplasmas without common bacteria, bronchitis or bronchiolitis and catarrhal bronchopneumonia were seen with peribronchial or peribronchial lymphoid hyperplasia, suggesting that severe pneumonia including supplicative or fibrous pneumonia may result from the secondary participation of common bacteria isolated frequently in such lesions.

Since Gourlay [1] isolated T-mycoplasmas from pneumonic lesions, the organisms have been suspected of a primary cause of calf pneumonia, while their exact role in pathogenesis remains unclear [6, 8]. Recently in Japan, the occurrence of this kind of pneumonia was reported, and T-mycoplasmas were recovered from those lesions at high incidence [10], although the etiology and pathogenesis of the disorders are still not fully understood, especially because of involvement of organisms other than mycoplasmas.

The present studies were performed to reveal histopathology of spontaneous cases of pneumonia in cattle and calves in special reference to bacteriological examination reported separately [12].

Materials and Methods

A total of 48 adult cattle and calves with pneumonia were collected in Miyagi and Tochigi Prefectures during a time period from April 1973 to March 1974. They were mostly of Holstein breed while some were of Japanese Black or Brown breed. Ages of animals were distributed widely, from 1 month to 6 years of age, but mostly less than 2 years of age (Table 1). After gross observation of pneumonic lesions, portions of lung tissues including macroscopic lesions were fixed in 10% neutral buffered formalin, embedded in paraffin, and 5 or 6 μm sections were prepared. Histopathological observations were made on sections stained with hematoxylin and eosin, Masson's trichrome, elasta-
Table 1. Age distribution of cases examined

<table>
<thead>
<tr>
<th>Age in month</th>
<th>No. of cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 6</td>
<td>15 (31%)</td>
</tr>
<tr>
<td>7–12</td>
<td>7 (15%)</td>
</tr>
<tr>
<td>13–24</td>
<td>14 (29%)</td>
</tr>
<tr>
<td>25≤</td>
<td>12 (25%)</td>
</tr>
<tr>
<td>Total</td>
<td>48 (100%)</td>
</tr>
</tbody>
</table>

van Gieson, periodic acid Schiff, Mallory's phosphotungstic acid-hematoxylin, MacCallura-Goodpasture and others.

Results

Gross observations

As shown in Fig. 1, regardless of age, pneumonic lesions were mostly distributed bilaterally from the marginal to the central areas of each lobe, most frequently at the anterior lobes. They were of various extent and well circumscribed having reddish or dark reddish color. Watery exudate was seen on the cut surfaces of some lesions while others showed sclerosis and collapse. Small abscesses also existed. In some cases fibrinous exudate on the pleural surfaces or thickening of interlobular septa was present. Pneumonic lesions were severer in cases under 6 months of age.

Histopathology

Histopathologically, different types of pneumonia were recognized (Table 2), and they were classified into 4 types as follows: 1) bronchitis or bronchiolitis of a limited degree, 2) catarrhal bronchopneumonia, 3) suppurative bronchopneumonia, 4) others including fibrinous pneumonia, interstitial pneumonia and atelectasis. The most frequent was lobular catarrhal bronchopneumonia, followed by suppurative broncho-pneumonia, bronchitis or bronchiolitis and others in this order of higher incidence.

Fig. 1. Distribution of gross lesions in the bovine lungs in different age-groups

≤ 6 months  | 7 - 12 months

13 - 24 months  | 25 months ≤

Remarks.

● : Pneumonic lesions.
● : Abscesses.
### Table 2. Histopathology of pneumatic lungs

<table>
<thead>
<tr>
<th>Age in months</th>
<th>Type of pneumonia</th>
<th>Number of cases</th>
<th>Bronchitis or Bronchiolitis</th>
<th>Alveolitis</th>
<th>Giant cells</th>
<th>Lymphoid hyperplasia</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 6</td>
<td>Catarhal bronchopneumonia</td>
<td>7</td>
<td>7 (+~+++)*</td>
<td>7 (+~+++)*</td>
<td>6 (+~+++*</td>
<td>5 (+~+++*</td>
</tr>
<tr>
<td></td>
<td>Suppurative bronchopneumonia</td>
<td>7</td>
<td>7 (+~+++*</td>
<td>7 (+~+++*</td>
<td>3 (+)</td>
<td>3 (+~+++*</td>
</tr>
<tr>
<td></td>
<td>Fibrinous pneumonia</td>
<td>1</td>
<td>0</td>
<td>1 (++)</td>
<td>1 (+)</td>
<td>0</td>
</tr>
<tr>
<td>7–12</td>
<td>Bronchitis or Bronchiolitis</td>
<td>2</td>
<td>2 (+~+++*</td>
<td>0</td>
<td>2 (+)</td>
<td>2 (+~+++*</td>
</tr>
<tr>
<td></td>
<td>Catarhal bronchopneumonia</td>
<td>3</td>
<td>3 (+~+++*</td>
<td>3 (+)</td>
<td>3 (+~+++*</td>
<td>3 (+~+++*</td>
</tr>
<tr>
<td></td>
<td>Suppurative bronchopneumonia</td>
<td>1</td>
<td>1 (+)</td>
<td>1 (+)</td>
<td>1 (++)</td>
<td>1 (+)</td>
</tr>
<tr>
<td></td>
<td>Interstitial pneumonia</td>
<td>1</td>
<td>0</td>
<td>1 (++)</td>
<td>1 (++)</td>
<td>0</td>
</tr>
<tr>
<td>13–24</td>
<td>Bronchitis or Bronchiolitis</td>
<td>4</td>
<td>4 (+~+++*</td>
<td>1 (+)</td>
<td>1 (++)</td>
<td>2 (+++*</td>
</tr>
<tr>
<td></td>
<td>Catarhal bronchopneumonia</td>
<td>6</td>
<td>6 (+~+++*</td>
<td>6 (+~+++*</td>
<td>3 (+~+++*</td>
<td>6 (+~+++*</td>
</tr>
<tr>
<td></td>
<td>Suppurative bronchopneumonia</td>
<td>3</td>
<td>3 (+~+++*</td>
<td>3 (+~+++*</td>
<td>1 (+)</td>
<td>3 (+)</td>
</tr>
<tr>
<td></td>
<td>Atelectasis</td>
<td>1</td>
<td>0</td>
<td>1 (+)</td>
<td>0</td>
<td>1 (+)</td>
</tr>
<tr>
<td>25≤</td>
<td>Bronchitis or Bronchiolitis</td>
<td>1</td>
<td>1 (++)</td>
<td>0</td>
<td>0</td>
<td>1 (++)</td>
</tr>
<tr>
<td></td>
<td>Catarhal bronchopneumonia</td>
<td>4</td>
<td>3 (+~+++*</td>
<td>4 (+~+++*</td>
<td>0</td>
<td>2 (+~+++*</td>
</tr>
<tr>
<td></td>
<td>Suppurative bronchopneumonia</td>
<td>2</td>
<td>2 (+~+++*</td>
<td>2 (+~+++*</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Fibrinous pneumonia</td>
<td>1</td>
<td>1 (+)</td>
<td>1 (++)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Pulmonary edema</td>
<td>1</td>
<td>1 (+)</td>
<td>1 (++)</td>
<td>0</td>
<td>1 (++)</td>
</tr>
<tr>
<td></td>
<td>Atelectasis</td>
<td>3</td>
<td>1 (+)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Remarks.

*: Number of cases and severity of lesions in parenthesis; (+) slight, (++) moderate, (+++) severe, (++++) very severe.

In a type, in which bronchitis or bronchiolitis was the most predominant change, there were degeneration, necrosis and erosion of the epithelium with neutrophil and macrophage accumulation in the lamina propria, and sometimes a mass of pus was present in bronchial or bronchiolar cavities. Lymphocytes and plasma cells were also infiltrated diffusely in the lamina propria, where fibrous proliferation occurred, occasionally projecting into the bronchial cavities (Figs. 2–4). In addition to these lesions, peribronchial or peribronchiolar lymphoid hyperplasia was seen in most cases, showing sometimes nodular appearances or formation of lymphoid follicles (Fig. 5).

In another type showing predominantly catarrhal bronchopneumonia, alveolar cavities were filled with albuminous materials, macrophages, lymphoid cells, polymorphonuclears and eroded epithelial cells (Fig. 6). Alveolar walls were hyperemic or swollen. Giant cells were seen in some cases, and they were mostly of foreign body type, while a few were of syncytiat type. In another type characterized by suppurative bronchopneumonia, a number of neutrophils and their debris were accumulated in the bronchial, bronchiolar and alveolar cavities, sometimes forming microabscesses into adjacent lung tissues (Fig. 7).

In addition to these three types of pneu-
monia, just described, there were two cases of fibrinous pneumonia (Fig. 8) and one case of interstitial pneumonia. In the latter, alveolar epithelial cells were proliferated showing cuboidal metaplasia and the walls were thickened with increased cellularity (Fig. 9). Atelectasis was also seen without any inflammatory reactions. In some cases with severe pneumonia, edema, hemorrhage and cellular infiltration were observed with dilatation of lymphatic vessels and hyperplasia of connective tissues in the alveolar septum.

The above described characteristics represented only main ones, and various types of lesions were frequently mixed within a single lesion in most cases. In most of young animals, fibrinous exudate or organization of them was also associated.

When these types of lesions were considered in relation to each age group, most cases under 6 months of age were found to have catarrhal and suppurative bronchopneumonia, as presented in Table 2. Especially about a half of this age group were of the latter. In contrast, suppurative bronchopneumonia was less frequent in older age groups and catarrhal bronchopneumonia was seen in about 48% of animals 7 to 24 months of age. Also bronchitis or bronchiolitis was seen in about 30% of animals 7 to 24 months of age, while other types of pneumonic lesions were predominant in animals over 25 months of age.

As described above, in animals under 6 months of age, more severe pneumonia was seen in accordance with gross observations, while lesions were less severe with only poor inflammatory reactions in those over 7 months of age, suggesting significant correlation between age factors and the types of pneumonic lesions.

In most cases described above, peribronchial or peribronchialolar lymphoid hyperplasia was seen irrespective of pneumonic types, and its incidence was as high as 86% in the age groups of 7 to 24 months whereas 50% to 60% in those either under 6 months or over 25 months of age. Interestingly, such lymphoid hyperplasia was shown to be associated most frequently with catarrhal bronchopneumonia except for the age group of 25 months or over.

Relations between pathological features of pneumonic lesions and organisms isolated

T-mycoplasmas were isolated at a high incidence from pneumonic lesions of the cases examined [12]. From the group under 6 months of age, T-mycoplasmas were isolated from 90%, and Pasteurella multocida was also isolated at a rather high incidence.

Relations between isolation of either mycoplasmas or common bacteria and histopathological features are presented in Table 3. Of cases having only mycoplasmas, bronchitis or bronchiolitis was seen in 20%, while catarrhal bronchopneumonia in 60%. Of cases having both mycoplasmas and common bacteria, suppurative bronchopneumonia was seen in 44%. On the other hand, catarrhal bronchopneumonia was seen in 50% of cases from which only common bacteria were detected. In cases having neither mycoplasmas nor common bacteria, other types of lesions including interstitial pneumonia and atelectasis were seen in 71%. While 80% of cases having only mycoplasmas showed lymphoid hyperplasia, its specific relations to isolation of T-mycoplasmas were not clear.

In 3 cases with suppurative bronchopneumonia, some bacteria were demonstrated also in sections. In 4 cases, fungi were detected in the bronchial cavities, while their etiological roles remained unknown. Eosinophilic intranuclear or cytoplasmic inclusion bodies suggesting viral infections were seen in the bronchial epithelium of a
Table 3. Relations between bacteriology and histopathology of pneumonic lungs

<table>
<thead>
<tr>
<th>Isolated organisms</th>
<th>Number of cases</th>
<th>Type of pneumonic lesions</th>
<th>Lymphoid hyperplasia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mycoplasmas*</td>
<td>Common bacteria**</td>
<td>Bronchitis or Bronchiolitis</td>
<td>Catarhal bronchopneumonia</td>
</tr>
<tr>
<td>+</td>
<td>10 (21%)</td>
<td>2***</td>
<td>6</td>
</tr>
<tr>
<td>+</td>
<td>23 (48%)</td>
<td>4</td>
<td>9</td>
</tr>
<tr>
<td>+</td>
<td>8 (17%)</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>=</td>
<td>7 (14%)</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

Remarks.

*: T-mycoplasmas, M. boviscinis and/or M. bovigenitalium.

**: Pasteurella multocida, Corynebacterium pyogenes and/or Haemophilus spp.

***: Number of cases.

few cases under 6 months of age.

**Discussion**

Most cases of pneumonias were of lobular type, and they were distributed mainly in the bilateral anterior lobes. Histopathologically, most of them were shown to be bronchopneumonia in accordance with Gourlay et al. [2]. Histopathological changes which were the most characteristics of bronchopneumonia, were 1) degeneration, necrosis and erosion of the bronchial or bronchiolar epithelium, 2) fibrous proliferation in the lamina propria associated with lymphoid cell infiltration, and 3) lymphoid hyperplasia in peribronchial or peribronchiolar areas. The latter two may be considered as rather chronic changes following bronchitis, bronchiolitis or catarrhal bronchopneumonia and sometimes with suppurative bronchopneumonia. Peribronchial fibrous proliferation and lymphoid hyperplasia were seen neither in fibrinous nor interstitial pneumonia. In reference to the results of bacteriological examination [12], suppurative or fibrinous lesions might have been caused as a result of mixed infection with various kinds of bacteria. Edema of alveolar septum with dilatation of lymphatic vessels was seen in a few cases, but it was not considered as characteristic lesions in calf pneumonia.

Lymphoid hyperplasia was seen in many cases regardless of difference in age and types of pneumonia, but incidence was the highest in catarrhal bronchopneumonia. Such lymphoid hyperplasia resembles that observed in swine enzootic pneumonia (SEP) or mycoplasma pneumonias in other animals [5,7,11]. Gourlay et al. [2] described similar findings in calf pneumonia. However, it is notable that such lesions were also recognized in some of the present cases from which no mycoplasmas could be demonstrated.

Giant cells seen in some of our cases were mostly of foreign body type while a few were of syncytial type as reported by Gourlay et al. [2]. Inclusion bodies were not detected in such giant cells. Acidophilic inclusion bodies were found in epithelial cells of the bronchi or bronchioli in some cases under 6 months of age, but their significance in etiology remains unclear. Calves under 6 months of age had generally more severe lesions characterized by small abscesses and fibrinous exudate in the pleural surfaces with catarrhal or suppurative inflammation. In contrast, in many cases over 7 months of age bronchitis or bronchiolitis as well as mild catarrhal bronchopneumonia were seen to a limited degree. In animals
over 25 months of age, other types of pneumonia lesions were predominant, and most of them were of mild and chronic ones. Thus, difference in characteristics of lesions was obvious among age groups.

With such differences in pneumonia types with age groups, 1) difference in pathogenesis, 2) difference in developmental stages of pneumonia, 3) age difference in resistance of host animals, should be taken into consideration. Since almost similar organisms were isolated in each group [12], each pneumonia type cannot be attributed to specific bacterial species. On the other hand, since various kinds of organisms were isolated and each animal was kept in different environments, it is difficult to consider that each type of pneumonia represents different stages of the same course. The most possible may be that difference in pneumonia types results from difference in resistance among individual animals particularly among age groups. The most severe pneumonia might have been caused in calves under 6 months of age by mixed infections with mycoplasmas and common bacteria.

From the present cases T-mycoplasmas were isolated at the highest incidence [12]. Of animals under 6 months of age, they were isolated in 90%, suggesting important role of T-mycoplasmas in producing calf pneumonia. However, in those younger animals, common bacteria, for example, Pasteurella multocida were also isolated at rather high incidence, and severe suppurative bronchopneumonia was prominent in many cases of this group. From the cases 7 to 24 months of age, most of which showed catarrhal bronchopneumonia, bronchitis or bronchiolitis, common bacteria were also isolated. On the contrary, in the group over 25 months of age having mild lesions, either mycoplasmas or common bacteria were isolated at lower incidence. Thus, severe pneumonia was seen in many cases under 6 months of age from which many species of bacteria were isolated, while mild pneumonia was seen in many cases from which a few species of bacteria were isolated. Consequently, the incidence of isolation of common bacteria is correlated with the severity of lesions to some extent.

When the presence of mycoplasmas and common bacteria in the lesions was compared to types of pneumonia, catarrhal bronchopneumonia, bronchitis or bronchiolitis appeared in many cases from which only mycoplasmas were isolated, as shown in Table 3. Contrary to this, suppurative bronchopneumonia was seen in many cases from which both mycoplasmas and common bacteria were isolated. Therefore, it is possible that mild inflammation might be due to mycoplasmas, while severe pneumonia with suppurative inflammation results from mixed infections with common bacteria. However, it remains unknown whether either T-mycoplasmas or large colony mycoplasmas (M. bovirhinis and M. bovigenitalium) would be related more closely to specific types of pneumonia. Gourlay and Thomas [3, 4] showed that milder pneumonia was produced experimentally by T-mycoplasmas and that the lesions were limited to peribronchial or peribronchiolar areas, indicating a role of T-mycoplasmas as a primary pathogene of calf pneumonia.

Acknowledgments: The authors wish to express their gratitude to Drs. H. Nakane, U. Muramatsu and T. Ogata, of Animal Health Laboratory, Utsunomiya, Tochigi, and Drs. A. Orii, M. Suzuki and M. Sugawara, of Sendai Meat Inspection Laboratory, Sendai, Miyagi, for their sampling materials examined.
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References


Explanation of Figures

Fig. 2. Bronchitis and bronchiolitis in a case 13 months of age having only T-mycoplasmas. Hematoxylin and eosin (H-E) stain.

Fig. 3. Bronchitis associated with infiltration of lymphoid cells in the lamina propria from a case 13 months of age having only T-mycoplasmas. H-E stain.

Fig. 4. Bronchitis associated with fibrous proliferation in the lamina propria from a case 24 months of age having only common bacteria. H-E stain.

Fig. 5. Lymphoid hyperplasia in the peribronchial area from a case 5 months of age having mycoplasmas and common bacteria. H-E stain.

Fig. 6. Catarrhal bronchopneumonia in a case 20 days of age having only T-mycoplasmas. H-E stain.

Fig. 7. Suppurative bronchopneumonia associated with microabscesses in a case 6 months of age having mycoplasmas and common bacteria. H-E stain.

Fig. 8. Fibrinous pneumonia in a case 1 months of age having mycoplasmas and common bacteria. Mallory’s phosphotungstic acid and hematoxylin stain.

Fig. 9. Interstitial pneumonia associated with thickening of alveolar walls with increased cellularity in a case 10 months of age having neither mycoplasmas nor common bacteria. H-E stain.

Bar=50 μm