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Abstract: One hundred pneumonic lungs from calves were examined by an immunoperoxidase technique using avidin-biotin-peroxidase complex method in formalin-fixed, paraffin-embedded sections for *Haemophilus somnus*. In the histological examination of tissue sections, strong positive reactions against *H. somnus* were detected in 14% of the lungs. *H. somnus* antigens were observed at the surface and/or within the epithelial cells, macrophages, necrotic alveolar walls, alveolar-bronchial/bronchiolar exudates, fibrins, distended interlobular septa and perivascular areas. In addition, dense antigen accumulations were detected in the necrotic macrophages and neutrophils.

Key Words: *Haemophilus somnus*, Immunoperoxidase, Pneumoniae.

Haemophilus somnus Antijeninin İmmunoperoksidaz Tekniği Kullanılarak Pnömonik Sığır Akciğerlerinde Lokalizasyonu

Özet: Pnömonili 100 buzağının akciğerleri immunoperoksidaz tekniklerden olan avidin-biotin peroksidaz kompleks metodu kullanılarak *Haemophilus somnus* bakımından incelendi. Test formalinde tespit edilmiş parafine gömülmüş doku kesitlerine uygulandı. Doku kesitlerinin histolojik incelenmesinde *Haemophilus somnus*'a karşı oluşan güçlü pozitif reaksiyon akciğerlerin %14'ünde saptandı. *Haemophilus somnus* antijenleri; epitel hücrelerinin yüzeyinde ve/veya içinde, makrofajlarda, nekrotik alveol duvarlarında, alveol-bronş/bronşiollerdeki eksudatlarda, alveol lumenlerindeki fibrinlerde, genişlemiş interlobüler septumlar ile perivasküler bölgelerde gözlendi. Ayrıca, yoğun antijen birikimleri nekrotik makrofaj ve nötrofillerde de görüldü.

Anahtar Sözcükler: *Haemophilus somnus,* immunoperoksidaz, pnömoni.

Introduction

Haemophilus somnus infection in cattle was recognized first as an infectious disease affecting the central nervous system. However, several other syndromes in cattle (genital tract diseases, myocarditis, polyarthritis, septicaemia, weak calf syndrome) have been associated with infection by this organism (1). At the same time, *H. somnus* is also an important cause of bovine pneumonia (2). This pathogenic potential of *H. somnus* and the economic importance of the above diseases indicate that this organism is a significant bovine bacterial pathogen.

Pathological (3, 4) and immunohistochemical findings (5) in pneumonias experimentally induced by *H. somnus* have been reported. Descriptions of *H. somnus* experimental pneumonia suggest that the lesions are similar to those seen in field cases (3, 5). However, there are few detailed studies of natural pneumonia associated with *H. somnus*, and these studies have concentrated on pathological (6,7) and relatively immunocytochemical findings (8) of the infection on the occasion of retrospective features. The present paper describes immunohistochemical and microbiological findings of pneumonic bovine lung associated with *H. somnus*. The

distribution and localization of the bacteria in pneumonic lung sections detected by immunoperoxidase technique are also described.

Materials and Methods

From March 1995 through June 1996, 100 pneumonic lungs from beef calves of 12-16 months old were obtained from a local abattoir. Samples of lung tissue were taken from pneumonic regions located usually in the right cranial lobes. The tissue sections were fixed in 10% buffered neutral formalin and processed by conventional methods and embedded in paraffin wax.

The avidin-biotin-peroxidase complex (ABPC) procedure was applied as previously described (9, 10). The reagents used (except the primary antiserum) were of commercial origin (Vectastain kit, Vector Laboratories Inc, Burlingame, USA). *H. somnus* strain Sz 1224, kindly provided by Dr. W. Haastra (Regional Animal Health Center, Boxtel-Holland), was used to produce primary antisera in rabbits. Primary antisera against *H. somnus* had titers of 1:2560 by tube agglutination test.

The ABPC procedure was performed according to the manufacturer's directions. Processed sections were incubated sequentially with normal goat serum, diluted primary antisera (1:640 working dilution), biotinylated anti-rabbit IgG goat serum and ABPC reagent. The peroxide was localized with AEC chromogen, then sections were counterstained with Mayer's hematoxylin.

Lung sections from which *H. somnus* was or was not isolated were used as positive and negative controls. Control sections were also incubated with either nonimmune serum or saline solution.

In the bacteriological examination (n: 100), the lung homogenates were cultured on brain-heart infusion agar containing 5 % defibrinated sheep blood and 0.5 % yeast extract and were incubated in 5 % CO_2 at 37 °C for 48-96 hours. *H. somnus* was identified according to the criteria described previously (11).

Results

Histopathological and other bacterial findings were reported previously (12).

Using an ABPC immunoperoxidase technique, *H. somnus* antigen was detected in formalin-fixed, paraffin-embedded pneumonic bovine lung. The distribution and localization of *H. somnus* antigens had a close association with the duration of the lesions. Antigen was commonly detected in the surface and cytoplasm of bronchial/bronchiolar epithelial cells, within the cytoplasm of intact alveolar macrophages in the subacute and/or chronic pneumonia (proliferative pneumonia). In addition to these localizations, in acute pneumonic cases, immunopositive materials were mainly observed in the necrotic alveolar walls, alveolar-bronchial/bronchiolar exudates (Figs. 1 and 2), fibrins, distended interlobular septa, perivascular areas and, were not seen in vessel walls. Dense antigen accumulations were also detected in the necrotic macrophages and neutrophils. *H. somnus* was isolated mainly from the acute exudative-proliferative pneumonia and a strong reaction was also detected using

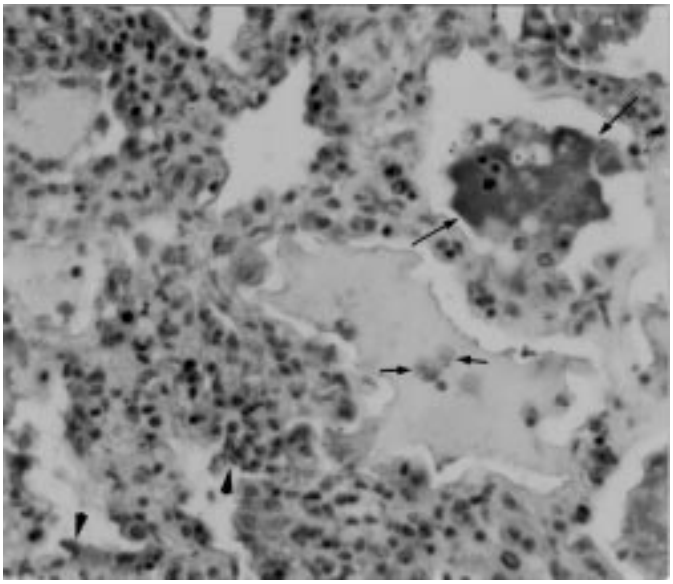

Fig. 1. Immunoperoxidase staining of pneumonic lung. Bacteria are seen clumped in alveoli (arrow), macrophages (small arrows) and in the epithelial cells on alveolar wall (arrowheads) Avidin-biotin peroxidase complex method. Mayer's haemotoxylin counterstain. x 100.

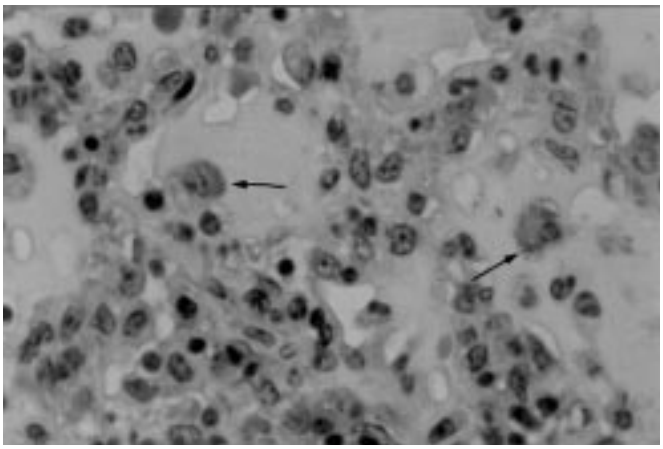

Fig. 2. Specific reaction against *H. somnus* in alveolar macrophages (arrows). Avidin-biotin peroxidase complex method. Mayer's haemotoxylin counterstain. x 380.

the IP test at the same aspect of pneumonia. *H. somnus* could not be isolated from the proliferative pneumonia. In this kind of pneumonia, the antigen seen in macrophages probably was attributable to phagocyted bacteria.

Specific staining was absent in the negative control tissue sections and test sections using nonimmune rabbit serum and saline solution instead of primary antiserum. Some nonspecific staining of connective tissue persisted. However, this staining could be differentiated from specific staining and did not confuse clarification.

Discussion

The localization of *H. somnus* in the subacute and/or chronic pneumonia also proved that chronic persistent infection occurs in calves (13). This study also confirms the predominantly extracellular nature of *H. somnus* (5, 8) and also localization of *H. somnus* in the greatest tissue damage areas (5) using an IP test. Diffuse specific reaction against *H. somnus* in those areas probably reflected the deposition of solubilized bacterial antigen that resulted from the degeneration and destruction of the bacteria.

Bacterial isolation from pneumonic lung tissue did not always agree with results obtained by the IP test for bacterial antigen. The discrepancy seems to be due simply to a difference in the sensitivity of the two detection systems. Furthermore, the four cases with strong positive IP and negative culture may be explained by the presence of antigen from live-dead and antigenic structures of bacteria whereas bacteriological cultures do not, obviously, detect dead and/or destroyed organisms especially in proliferative pneumoniae. In view of all these facts, the IP technique has the advantage of the detection of dead and/or low numbers of bacteria and, antigenic materials.

An immunoperoxidase technique appropriate and reliable for paraffin sections can make an important contribution to the detection and study of *H. somnus* infection. The morphology is excellent and both positive and negative cells can be accurately classified and counted in this technique. Therefore, we considered that the IP test was the superior method to determine the correlation between histopathological findings and the causative organisms with their exact localization in tissues.

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