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Distribution and Morphological Study of the Bronchus Associated Lymphatic Tissues (BALT) in Three Months Old Calves

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Abstract: The distribution and morphological features of bronchus associated lymphoid tissue (BALT) were evaluated in three months old calves. Five types of lymphoid tissue were found but their distribution varied between regions and individuals. Scattered lymphoid cells were most predominant form. Dense lymphoid aggregates were frequently seen in bronchioles. Follicular lymphoid tissues were seen in bronchi. Small numbers of intraepithelial lymphoid cells were always located throughout the epithelium of mucosa. The intraluminal lymphocytes were observed mainly in bronchi and large bronchioles. The follicular development of BALT was less frequent and only occurred in the large bronchi of anterior cranial lobe in calves 3. The lymphoepithelium invagination of bronchi can be found in calve 1 as in that of duodenum of gut. The occurrence and morphology of BALT were different within the individual.

Key words: Histology, BALT, calves

Introduction

Infectious and hypersensitivity disease of lungs is important and common in calves. The respiratory tract represents an important component of the mucosal immune system and contributes in the protection of respiratory tract from respiratory pathogens. In other species, the mucosal immune system plays an important role in the protection of respiratory tract (McDermott *et. al.* 1982). The local immunity occurs independently without the presence of systemic humoral and cellular immunity. The lymphoid tissue associated with the bronchus had been investigated in relation to immunity and its structural and functional similarity to the Peyer`s patches had let to the suggestion that this local lymphoid tissue may form part of a common mucosal immune system (Bienenstock *et. al.*, 1973a; Bienenstock 1974). They have showed that BALT is a complex structure containing lymphoid follicles with adjacent mixed lymphoid cells and venules with high endothelium.

In human, BALT has been found under pathologic conditions such as chronic respiratory infection but it is not present in healthy adults (Pabst and Gehrke 1990). Thus, BALT is involved the mucosal immunity of human lungs under conditions such as chronic respiratory diseases, immunodeficiency, autoimmune diseases, panbronchiolitis and rheumatoid arthritis (Meuwissen and Hussain 1982; Rogers, *et. al.* 1986; Sato *et. al.* 1992). However, BALT has been observed in the bronchial wall of several mammalian species and may be analogous to gut associated lymphoid tissue. It consists of aggregates of lymphoid and non-lymphoid cells with specialized lymphoepithelium and is more frequently located at the bifurcation of the tracheobronchial tree. Differences in structures and functions have been observed between the different species.

The objective of this study was to describe the distribution and morphological features of BALT in the non-pneumonic lungs of three months old calves and their comparison between individual.

Materials and Methods

Three calves of three months old were use in this study. The calves were supplied by the University Research Park, Universiti Putra Malaysia. Upon slaughter the lungs were removed and examined grossly. The right lung was inserted and fixed with 10% neutral buffer formalin. The right anterior cranial lobe (ac), right posterior cranial lobe (pc) and right caudal lobe (ca) were taken for sampling. They were systematically trimmed at every 0.5cm length and samples were collected at 6 sites along the bronchus. The samples of lung tissues were embedded in paraffin, sectioned at 4 μm and stained with Haematoxylin and Eosin.

The sections of bronchus lymphoid tissue surrounded the selected bronchi were examined under light microscope and classified either as nodular or aggregates form before the cells were counted. The size of each BALT was determined using an image analyzer (PC IMAGE version 2.2, NewCastle Upon Tyne, U.K.). Significant differences between BALT of individual were calculated and analysed statistically using Ducan`s multiple range test.

Results

The number and types of BALT foci occurred within various airways. Variable amount of lymphoid tissues were

identified within individuals. The major lymphoid tissues found were in lymphoid aggregates form. The numbers

Table 1: The number of lymphoid aggregates of BALT in different lobes of each calve

	Anterior cranial Lobe Mean ± SEM	Posterior cranial Lobe Mean ± SEM	Caudal loab Mean ± SEM
Calve 1	813.1 ± 39.5^a	480.0 ± 51.5^b	81.9 ± 10.0^c
Calve 2	773.6 ± 56.2^a	433.2 ± 34.9^b	60.0 ± 4.1^c
Calve 3	665.7 ± 37.1^a	660.5 ± 44.8^a	54.4 ± 4.6^b

of lymphoid aggregates of BALT in different lobes of each calve and occurrences (Mean ± SEM) of lymphoid aggregates compared with each lobe in individual are showed in Table 1 and Fig. 1 respectively. There were significant differences ($p<0.05$) in the number of lymphoid aggregates between the three lobes (right anterior cranial lobe, right posterior cranial lobe and right caudal lobe) in calve 1 and calve 2. In calve 3, although the difference was not significant between the anterior and posterior cranial lobes, the number of lymphoid aggregates of caudal lobe was significantly ($p<0.05$) lower than the other two lobes. The number of BALT was higher in the cranial lobe. The dorsal part of caudal lobe had lowest number of lymphoid aggregates.

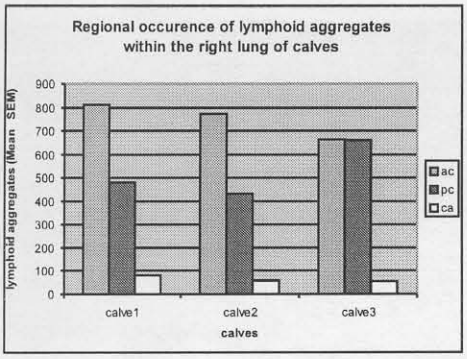
The mucosa lymphoid tissue could be subdivided into five different types: intraluminal lymphocytes, intraepithelial lymphocytes, scattered lymphoid tissues, dense aggregated lymphoid tissues and follicular lymphoid tissues (lymphoid follicles). Among them lymphoid follicles and unorganized lymphoid aggregates were commonly seen in the wall of small bronchi, between smooth muscle and cartilage plates (Figs. 2, 3 and 4). However, there is a different between individual. Calve 1 and calve 2 were found to have no evidence of lymphoid follicle in the right lung while calve 3 had lymphoid follicles in the anterior cranial lobe.

Intraepithelial lymphocytes (IEL) were found in all parts of bronchi to bronchioles. They were located at all depth of the epithelium but most numerous near the base of the epithelium (Fig. 5). IEL populations differ in several ways. Small number of neutrophil and eosinophil were often mixed with intraepithelial lymphocytes. The intraepithelial lymphocytes were most numerous in the overlying dense lymphoid aggregates and lymphoid follicles. Scattered small lymphocytes were commonly present in subepithelial sites in all area where lymphoid nodules were present (Fig. 6). They consist of predominantly small lymphocytes and often mix with small number of plasma cells and mast cells. Occasionally, eosinophils and neutrophils were also present. The cells were most numerous in the lamina propria adjacent to dense lymphoid aggregates and lymphoid nodules (Fig. 7), in region surrounding the submucosal gland (Fig. 8) and the mucosal epithelium (Fig. 9). Small lymphatic vessels were occurred among the lymphocytes.

Dense aggregated lymphoid tissues varied in size and were present around the bronchi and bronchioles (Fig. 10). Dense aggregated lymphoid tissues were frequently encountered beneath the muscularis. The aggregated cells were predominantly mononuclear cells of the lymphocytes, plasma cells series and sometimes with a few mast cells. The epithelium overlying the aggregates was normal and could be found the proliferation of epithelial cells (Figs. 11, 12 and 13). The epithelium of dense aggregated lymphoid tissues of bronchiole in calve 1 entered into the lymphoid aggregates and formed invagination (Fig. 14). The invaginated epithelium in the lymphoid aggregates was lined by cuboidal cells with intraepithelial lymphocytes (Fig. 15). Small lymphatic vessel was often present in the lamina propria and the area near to the large aggregates (Fig. 16). Small number of neutrophils and plasma cells were present in the interstitial connective tissue of lungs, especially in the area closed to blood vessels. Lymphoid aggregates were still common within the adventitia of bronchioles.

The lymphoid follicles were seen in the wall of bronchi of right cranial lobe in calves 3. Lymphoid follicles were structurally more complex than the lymphoid aggregates. Lymphoid follicles structurally similar to those in peripheral lymphoid nodes and other mucosa associated lymphoid tissue. On the basis of histological characteristics, the follicles could be divided into 4 different areas: follicular associated epithelium, dome area, follicles and inter-follicular area (Fig. 17). Follicular associated epithelium or lymphoepithelium was thinner than in adjacent area. The epithelium was composed of nonciliated flat cells, M cells and intraepithelial lymphocytes and was different from the adjacent respiratory epithelium. In the bronchi, the follicles occasionally formed a dome structure (Fig. 18). The large number of small lymphocytes occupied the intercellular spaces. Dome area contained small and large lymphocytes. The plasma cells were particularly numerous in the dome area where high endothelial venules were occasionally present. The follicles or nodules were composed of an uncapsulated ovoid mass of cells including small and medium lymphocytes and macrophages. Secondary follicles contained germinal center, which was almost completely surrounded by mantle zone. The mantle zone consisted of densely packed small lymphocytes with dark stained nuclei (Fig. 19).

The central paler zone consists of scattered macrophages, lymphocytes and dendritic reticular cells. Small number of lymphoid cells showed mitotic figures. The corona of densely packed small lymphocytes was thick nest on the epithelial aspect of the follicles. The inter-follicular area contained small lymphocytes, plasma cells, arterioles



ac - anterior cranial lobe pc - posterior cranial lobe
ca - caudal lobe

Fig. 1: Occurrence (Mean±SEM) of lymphoid aggregates compared with each lobe in individual

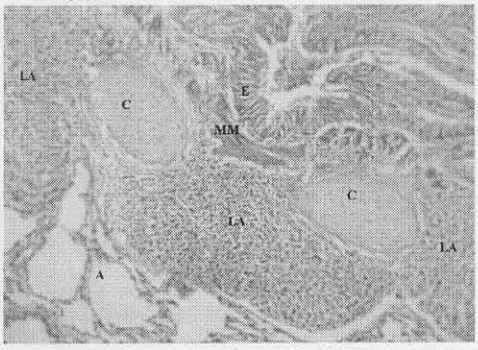


Fig. 2: Lymphoid agreegates of bronchi near the alveoli. MM, muscularis mucosa, C, cartilage, A, alveolus, E, epithelium, LA, lymphoid aggregate (x40)

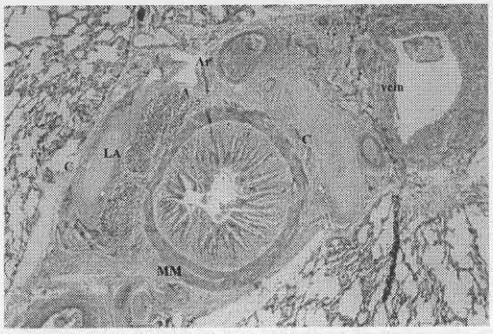


Fig. 2: Lymphoid agreegates within small bronchi occured external to the MM, muscularis mucosa, C, cartilage, Ar, arteriole, LA, lymphoid aggregate (x40)

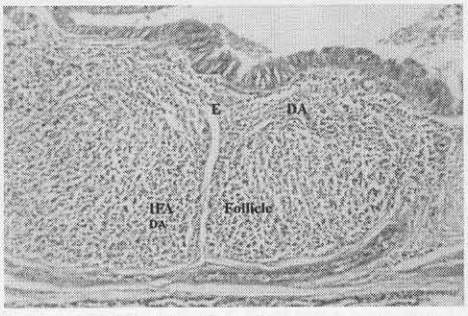


Fig. 4: Lymphoid follicles in he lamina propria of bronchi in calve 3. IFA, interfollicular area, E, epithelium DA, dome area (x100)

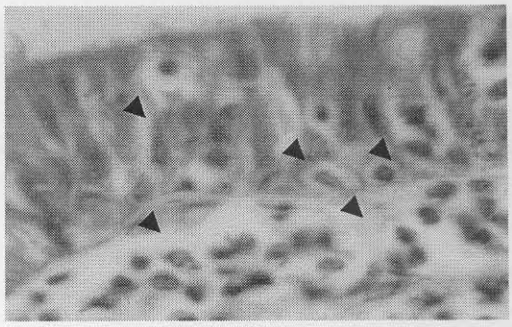


Fig. 5: Intraepithelial lymphocytes (arrows) within epithelium of bronchi (x 400)

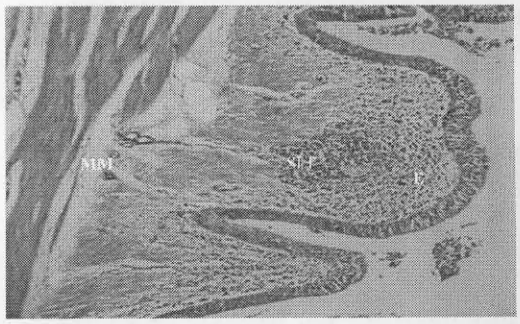


Fig. 6: Scattered lymphoid tissues (SLT) within the lamina propria in the large bronchi. MM, muscularis muscosa E, epithelium (x40)

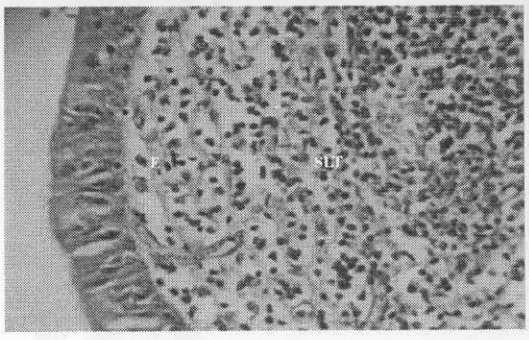


Fig. 7: Scattered lymphoid tissue (SLT) in the lamina propria\ of bronchus E, epithelium (x100)

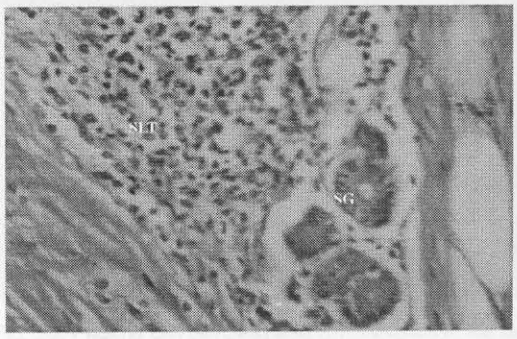


Fig. 8: Scattered lymphoid tissues (SLT) near the submucosal gland of large bronchi. SG, submucosa gland (x100)

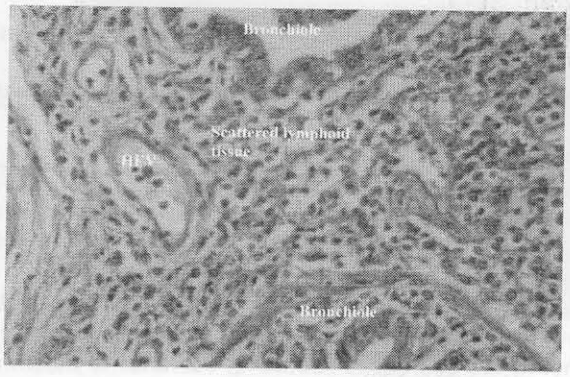


Fig. 9: Scattered lymphoid tissues beneath the epithelium of small bronchiole. HEV, high endothelial vanule (x100)

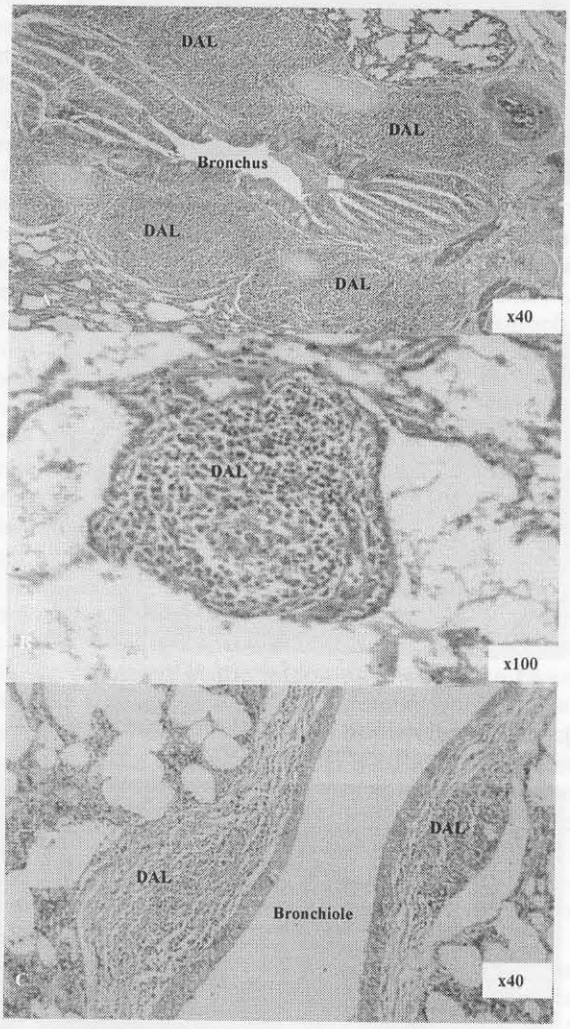


Fig. 10: Dense aggregated lymphoid tissues (DAL) of the bronchi (A) and bronchiole (B and C)

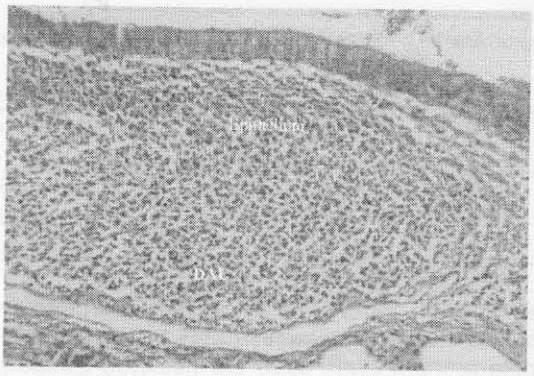


Fig. 11: Normal epithelium of dense aggregated lymphoid tissues (DAL) of Bronchiole (x100)

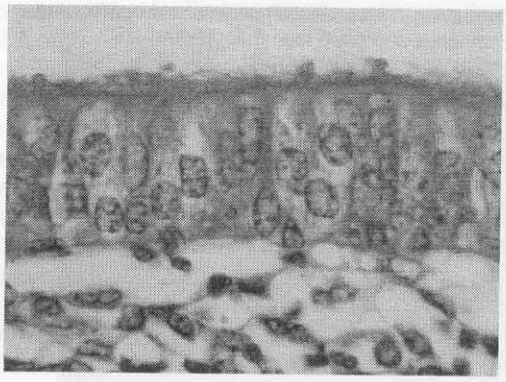


Fig. 12: Epithelium of dense aggregated lymphoid tissue of small bronchiole (x400)

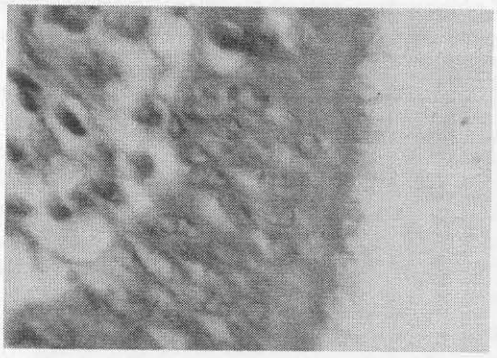


Fig. 13: Proliferation of epithelial cell of dense aggregated lymphoid tissue (x400)

Fig. 14: Invaginated epithleium of dense aggregated lyphoid tissue of bronchiole in calve 1 (x40)

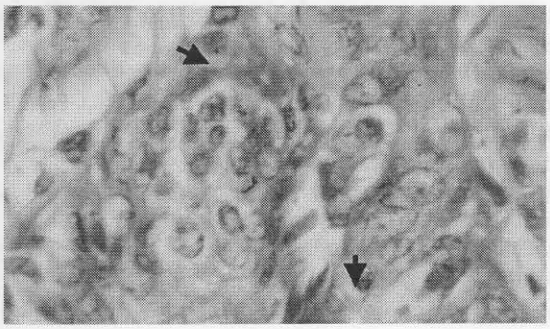


Fig. 15: High manification of invaginated epithelium of lymphoid aggregates. Arrows indicate the intraepithelial cells (x400)

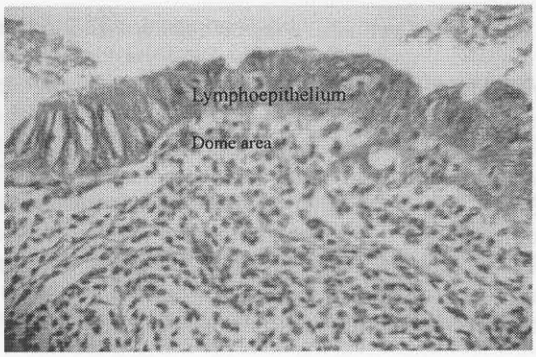


Fig. 18: Lymphoepithelium of large bronchi and dome area (x100)

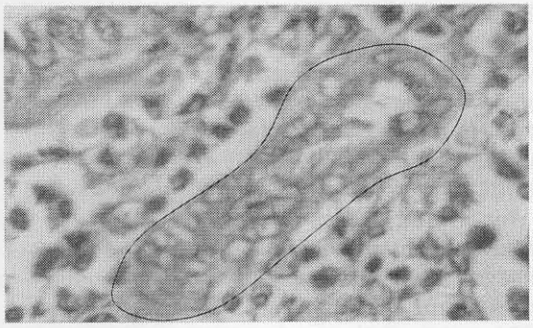


Fig. 16: Lymphatic vessel in the lamina propria of bronchi (x400)

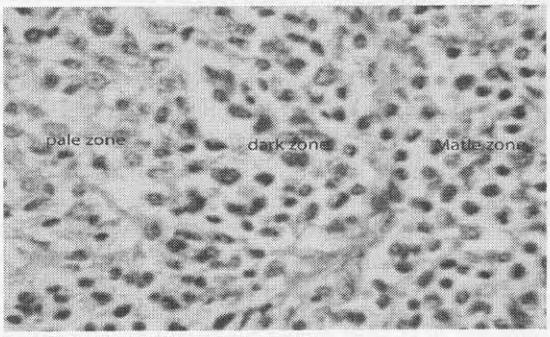


Fig. 19: Three different zones of the secondary lymphoid follicle of large bronchi (x400)

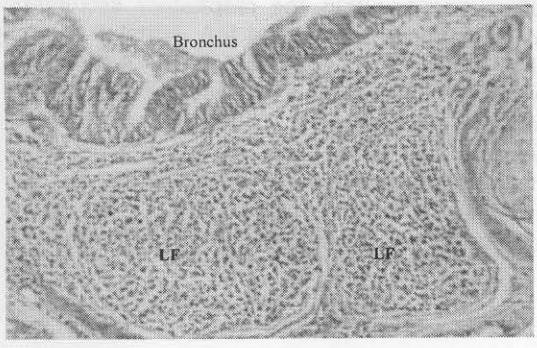


Fig. 17: Lymphoid follicle (LF) of large bronchi (x100)

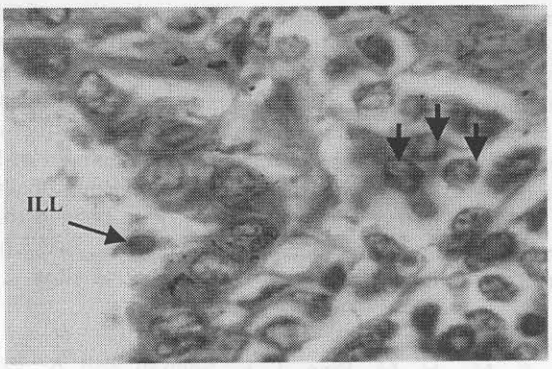


Fig. 20: Plasma cell (arrows) and lymphocytes in the lamina propria (x1000). ILL intraluminal lymphocyte

andhigh endothelial venules. Plasma cells were found centrally but were mostly present in the lamina propria (Fig. 20).

In the bronchioles, the lymphoid follicles were absent. Lymphiod follicles were interposed between bronchiole and blood vessel. Very few plasma cells were found in the alveolar or interlobular septa or pleura. The BALT was classified as lymphoid aggregates and lymphoid follicles but the lymphoid follicles were only seen in calves 3. The dorsal part of caudal lobe had significantly less ($p < 0.05$) lymphoid aggregates than anterior and posterior cranial lobes. BALT was located along the wall of airways. In large airways, the lamina propria and smooth muscle layer were prominent and the lymphoid nodules were predominantly present near the basement membrane of epithelium. Lymphoid aggregates were widely distributed throughout of airways in all region of the lung. Lymphocytes aggregates of bronchioles extended from the epithelium to adventitia. All regions contain BALT but there were greatest in anterior cranial lobe.

Discussion

The BALT in this study has similarity to the BALT described in animals and human lungs tissues (Bienenstock *et al.*, 1973a; Sminia *et al.*, 1989; Gould and Isaacson 1993; Tschemig *et al.*, 1995). BALT has been described to play a major role in the development of the local immune response to inhalated antigen in animals such as rabbits and rats and was similar to the Peyer`s patches in the gut (Bienenstock *et al.*, 1973a and 1973b). Bienenstock *et al.*, (1973a) described that BALT could be identified macroscopically in experimental animals including rabbits, rats, guinea pigs and chickens as white patches after acetic acid fixation. Plesch (1982) stated that 30 to 50 BALT aggregates appeared around the bifurcation of the major bronchus in adult rats macroscopically. However, in this study the BALT aggregation could not be seen macroscopically. There are significant differences between species in the degree of organization of BALT. In the present study, BALT distribution varied between the regions and individuals. Although 4 types of lymphoid tissue are morphologically similar to those of other species, it has been identified that the most predominant form is scattered lymphoid tissues (Mair *et al.*, 1987). The respiratory tract of healthy calves has dense lymphoid aggregates and less lymphoid follicles as compared to most species (Binenstock *et al.*, 1973a; Loo and Chin 1974 and Mair *et al.*, 1987). In this study, dense aggregations of lymphoid tissues were seen both in the bronchi and bronchiolar regions of the calves' lungs. This is similar to the finding in equine and ovine lungs (Mair *et al.*, 1987 and Chen *et al.*, 1989). The results of this study indicated that BALT consists predominantly of scattered lymphoid aggregates.

According to Anderson *et al.* (1986), the occurrence of BALT varied significantly according to the sites of lungs tissue. The observation in this study agreed with Anderson *et al.* (1986a). Bienenstock *et al.* (1973a) described that the lymphoepithelium overlying BALT was found in normal component of structure in mice. Anderson *et al.* (1986b) demonstrated that the lymphoepithelium was related to antigenic stimulation and pneumonic lesions. In this study, the lymphoepithelium was found in calve 3. Thus, the author's examinations of the lungs agree with Bienenstock *et al.* (1973a). The lymphoid tissues of this study may represent an early stage of developing BALT. They may grow as the result of antigenic stimulation of lungs. The lymphoepithelium may formed by direct contact with luminal antigens and immunocytes in the subepithelial lymphoid tissues. In this study the epithelium invagination of lymphoid aggregates of bronchiole were found in the bronchiole of cranial lobe. This finding was similar to the epithelium invagination of duodenal Peyer's patches.

Intraepithelial lymphocytes also occasionally found within epithelium in all parts of the bronchi and bronchioles. They were T lymphocytes (predominantly T helper cells) and may also play an important role in viral infection (David and Reinhard 2000). Mature plasma cells were present diffusely in the superficial layer of lamina propria and the mucosal glands. Their function is production of immunoglobulin present within the respiratory secretion. Immunoglobulin A is the major immunoglobulin in the reaspiratory secretion of cattle and IgA containing cells predominate in the bronchial mucosa and lung in these species (Allan *et al.*, 1979; Anderson *et al.*, 1986a).

The density of BALT varied between individual calves. Anderson *et al.* (1986a) indicated that respiratory tract associated lymphatic tissue is less abundant in normal cattle than in most other animals. They indicated that the occurrence and degree of the follicular development of BALT was quite variable within each of age group and local stimulation and environmental conditions. Further observation on the morphological development of BALT depends upon age and pathologic conditions. It is believe that the occurrence and development of BALT in calves is influenced by the pathological lesions of lungs. BALT plays an important role in the mucosal immunity of diseases affected the lungs by acting as their inductive sites.

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