J. Vet. Malaysia (2009) 21(2): 9 - 15

MORPHOLOGY OF LYMPHOID NODULES AND AGGREGATES IN THE LUNGS OF CALVES AT DIFFERENT AGES

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SUMMARY

The present study was conducted with the aim of defining the morphology of lymphoid nodules and lymphoid aggregates in calves at different ages. A total of 9 calves, divided into 3 age groups (3-, 6- and 8-month-old) consisting of 3 calves in each group, was used in this study. Samples of the lungs were taken systematically and processed for histological examinations. The tissues were sectioned at 4 μm, stained with Haematoxylin and Eosin and examined under light microscope. The occurrence and structure of lymphoid nodules and lymphoid aggregates varied between the age groups. The number of lymphoid nodules and lymphoid aggregates in the lung of calves increased with age. The number of lymphoid nodules and lymphoid aggregates in the 8-month-old calves was significantly higher ($P<0.05$) than in the 3- and 6-month-old calves. Similarly, the size of lymphoid nodule and lymphoid aggregates increased with age and was significantly higher ($P<0.05$) in the 8-month-old calves as compared to the 3- and 6- month-old calves. The number of intraepithelial lymphocytes (IELs) within the lymphoid nodules and lymphoid aggregates was significantly higher ($P<0.05$) in the 8-month-old calves compared to the 3- and 6-month-old calves.

Keywords: Morphology, lymphoid nodules, aggregates, lungs, calves, age

INTRODUCTION

Intrapulmonary lymphoid tissue can be found associated with the bronchus of animals. It is known as bronchus-associated lymphoid tissue (BALT) and is a member of a family of mucosa-associated lymphoid tissue (Bienenstock, 1984). The BALT consists primarily of a lymphoid cell compartment covered by an epithelium that is structurally different from typical bronchial epithelium. The BALT is complex and consists of lymphoid nodules and lymphoid aggregates (dense aggregated lymphoid tissues), scattered lymphoid tissues, intraepithelial lymphocytes (IEL) and interluminal lymphocytes. The BALT typically contains lymphocytes and antigen-presenting cells as well as high endothelial venules (Anderson *et al.*, 1986b). High endothelial venules are thought to be important in recirculation and homing of mucosa specific lymphocyte (Woodrufff *et al.*, 1987). Its function is initiation of a local immune response for luminal antigen. The M or microfold cell characteristic of BALT is a specialised epithelial cell engaged in active sampling of luminal content and capable of transfer of soluble and particulate matter bidirectional into or away from the lumen (Bockman and Steven, 1977). The BALT has been described previously in cattle (Anderson *et al.*, 1986a), mink (Jericho, 1970), rabbits, guinea pig, pig, rat, mice, dog, chicken and human (Bienenstock *et al.*, 1973). However, the morphology of BALT in calves in relation to age in a single study has never been reported and compared before. Thus, the present study reports the morphology of lymphoid nodules and aggregates at different sites of lungs of calves at different ages. In addition, the intraepithelial lymphocytes (IELs) within the lymphoid nodule and aggregates were also determined.

MATERIALS AND METHODS

Animals

A total of nine local breed calves of different ages (3-, 6- and 8-month-old) were used in this study. The calves were divided into three groups (n=3) according to age. The calves were supplied by the University Research Park, Universiti Putra Malaysia.

Tissue sampling and processing

The lungs were removed immediately upon slaughter and washed with phosphate buffer saline solution (pH 7.3). The right lung was infused with 10% buffered formalin via the right principle bronchus and allowed to fix for 24 to 48 hours before sampling. The samples were taken from 4 different lobes of the right lung namely the anterior cranial, posterior cranial, middle and caudal lobes. The samples were fixed in 10% buffered formalin and processed for histological examination. The tissues were embedded in paraffin, sectioned at 4 μm, stained with

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Haematoxylin and Eosin and examined under light microscope of up to 400 magnification.

Histological examination

The size of lymphoid nodules and aggregates was measured using the image analyser (Synoptic, Foster Finding Associated Ltd, Freepost NT 2042 Newcastle Technopole, UK). Within the lymphoid nodules and lymphoid aggregates, the intraepithelial lymphocytes (IELs) of epithelium were counted based on their morphological structure. The IELs with well defined nuclear were counted using the eyepiece gratitcule, and expressed as per 50 epithelial cells nuclei in the lymphoid nodules and lymphoid aggregates.

Statistical analysis

The data were analysed using the Statistical Analysis System and Duncan's multiple range test.

RESULTS

The lymphoid nodules were seen in the bronchi and bronchioles of the lung in all ages and were structurally more complex than the lymphoid aggregates. The lymphoid nodule consisted of dome area, follicle and corona. Primary and secondary follicles of the lymphoid nodule extended from the submucosa to direct contact with the basement membrane of the mucosal epithelium (Figure 1). The arterioles were present close to the lymphoid nodules. The lymphoid nodules were mainly composed of medium to large lymphocytes and a few reticuloendothelial cells, plasma cells and macrophages. The basic structures of lymphoid nodules were similar in all parts of bronchi, bronchioles and respiratory bronchioles and could be divided into four areas: the follicle, parafollicular area surrounding the follicle, lymphoepithelium and the dome area.

The lymphoid aggregates were present beneath the tunica muscularis and mostly found in tunica submucosa and lamina propria. They were located randomly in the bronchi and bronchioles. In 3-month-old calves, lymphoid aggregates were frequently seen in bronchioles. In 6- and 8-month-old calves, they were mostly found at the bifurcation of bronchi, bronchioles and terminal bronchioles (Figure 2).

The number of lymphoid nodules in lung increased with age (Figure 3). The number of lymphoid nodules in different lobes of lung was significantly different ($P<0.05$) between age groups. Within the group, the number of lymphoid nodules in different lobes of lung was also different. The number of lymphoid nodules in the cranial lobes (anterior and posterior) was significantly higher ($P<0.05$) than in the middle and caudal lobes in the 6- and 8-month-old calves. In 3-month-old calves, the lymphoid nodules were rarely present in the wall of bronchioles, and some part of the lobes had no evidence of lymphoid nodules. In 6-month-old calves, numerous well developed nodules were present near the opening of secondary bronchi in the anterior cranial lobes. In the 8-month-old calves, several large complex nodules were seen in the bronchi, bronchioles and respiratory bronchioles.

The length and width of lymphoid nodules in different lobes of lung of all age groups are shown in Figure 4. The size of lymphoid nodules increased with age. The length and width of lymphoid nodules of the anterior cranial lobe were significantly higher ($P<0.05$) than the other lobes of the lung. The length and width of

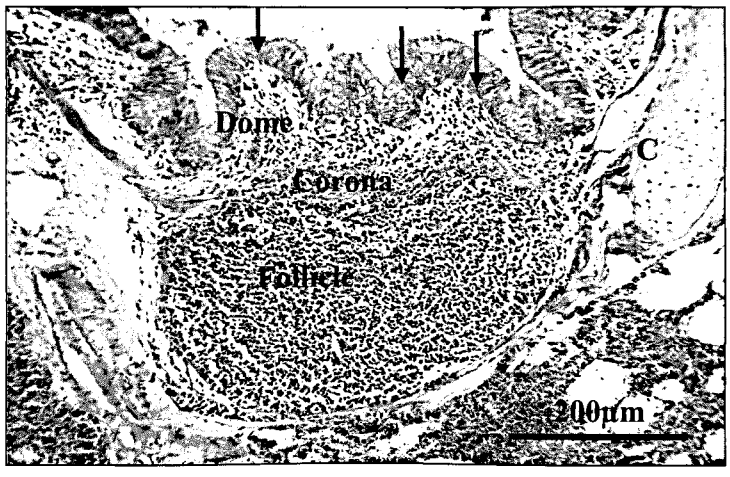

Figure 1: Histological section of lymphoid nodule at bronchial bifurcation in 8-month-old calf shows the nodule extends from the submucosa [containing cartilage (C)] to the basement membrane of the mucosal epithelium (arrows). The lymphoid nodule consists of dome area, follicle and corona. H & E.

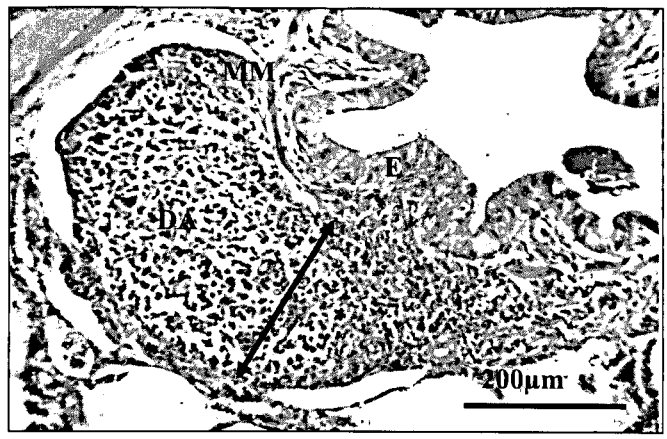

Figure 2: Dense aggregated lymphoid tissue (DA) in terminal bronchiole of a 6-month-old calf. It is located in the submucosa of a terminal bronchiole and the epithelium (E) overlying the aggregate is unspecialised. MM= muscularis mucosae. H & E

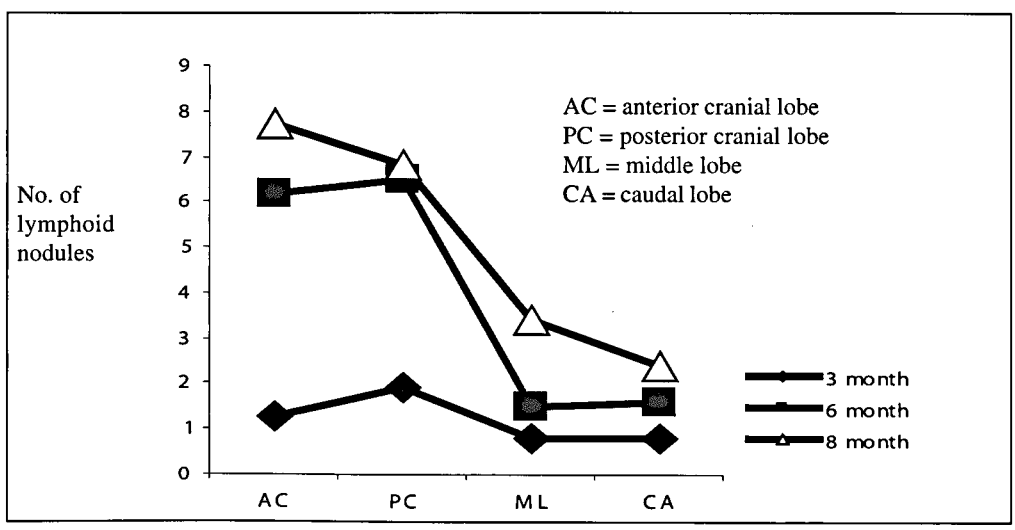

Figure 3: Number of lymphoid nodules in different parts of lung at different ages of calves

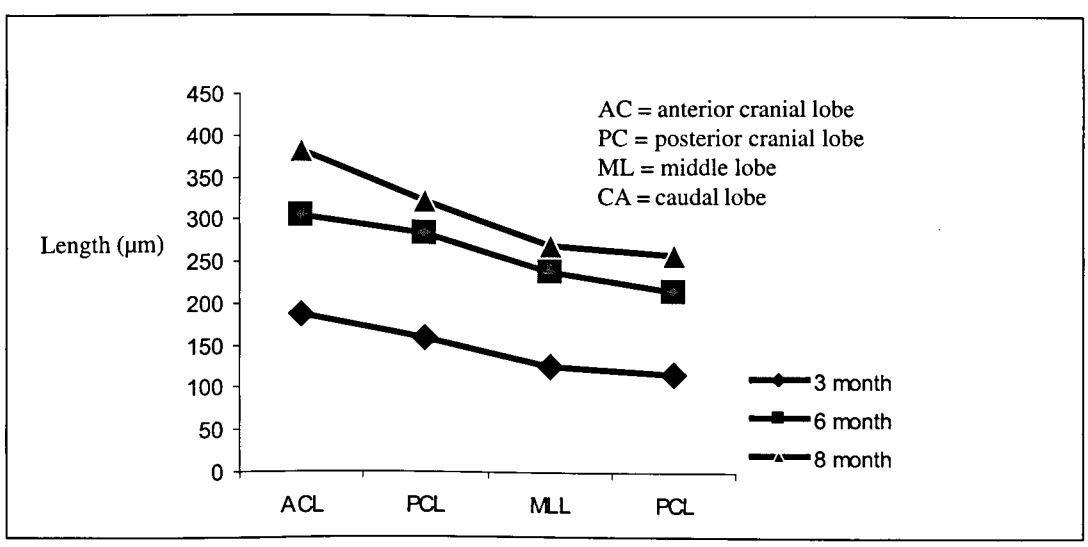

Figure 4A: Length of lymphoid nodules in different lobes of lung at different ages of calves

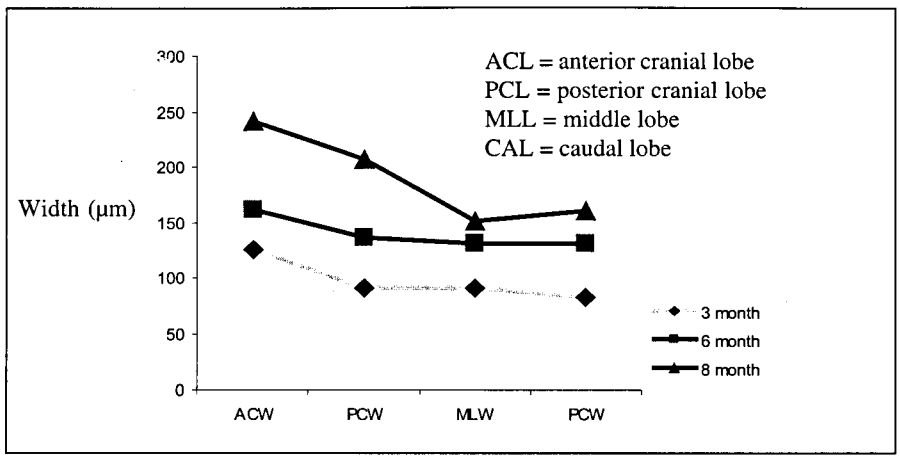

Figure 4B: Width of lymphoid nodules in different lobes of lung at different ages of calves

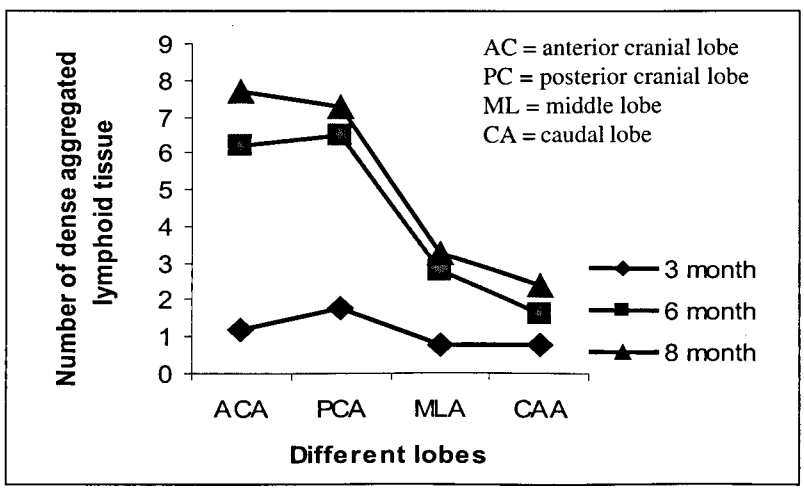

Figure 5: Mean number of lymphoid aggregates in different lobes of lung at different ages of calves

lymphoid nodules in 8-month-old calves was significantly higher ($P<0.05$) than in the 3- and 6- month-old calves.

The distribution of the lymphoid aggregates in different lobes of lung varied individually and between age groups. Figures 5, 6 and 7 show the number and size of lymphoid aggregates in different lobes of lung of all age groups. The number and size of the lymphoid aggregates increased with age. The number of lymphoid aggregates in the cranial lobe was higher than in other lobes (Figure 5). In the 3- and 6-month-old calves, the width of the lymphoid aggregates was not significantly different ($P>0.05$) within the same age (Figure 7). The width and length of lymphoid aggregates in 8-month-old calves were significantly higher ($P<0.05$) than in the 3- and 6-month-old calves.

Figure 8 shows the number of IELs in lymphoid nodules in different lobes of the lung in 3-, 6- and 8-month-old calves. The number of IELs in the cranial lobe was significantly higher ($P<0.05$) than in the other lobes; also the number of IELs increased with age. The number of IELs in lymphoid nodules in 8-month-old calves was significantly higher ($P<0.05$) than those of the 3- and 6-month-old calves. The number of IELs in lymphoid aggregates in the different lobes of the lung in the 3-, 6- and 8-month-old calves is shown in Figure 9. The number of IELs in lymphoid aggregates of the posterior cranial lobe was higher than in other lobes in 6 and 8 month-old calves. The number of IELs in lymphoid aggregates in 8-month-old calves was significantly higher ($P<0.05$) than in that of the 3- and 6-month-old calves.

DISCUSSION

The lymphoid tissues at different levels of the respiratory tract identified by our method are basically similar and histologically resemble those examined in other

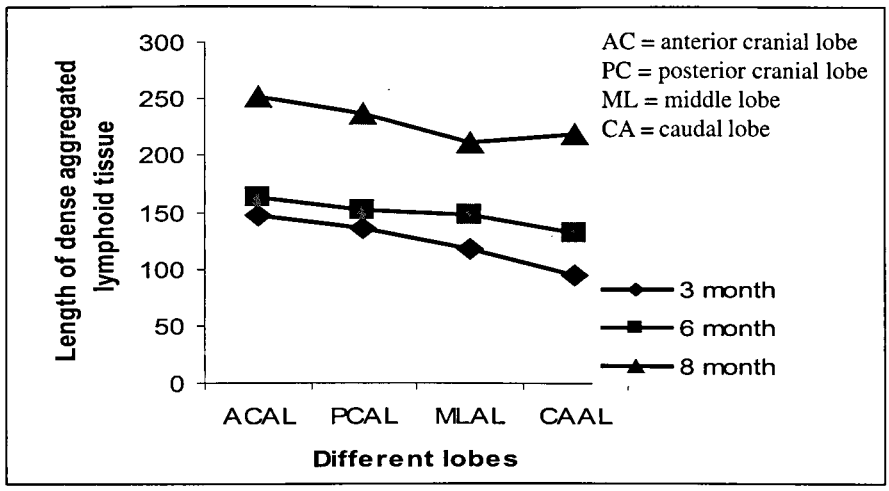

Figure 6: Length of lymphoid aggregates in different lobes of lung at different ages of calves

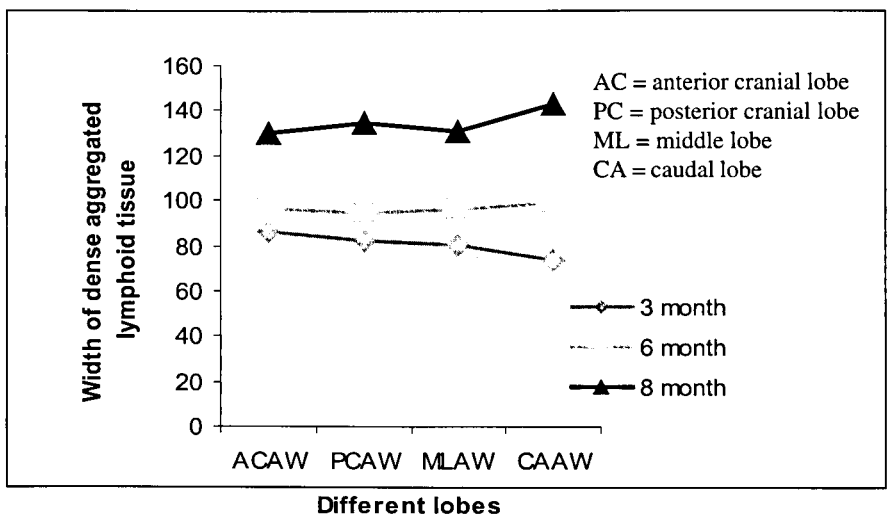

Figure 7: Width of lymphoid aggregates in different lobes of lung at different ages of calves

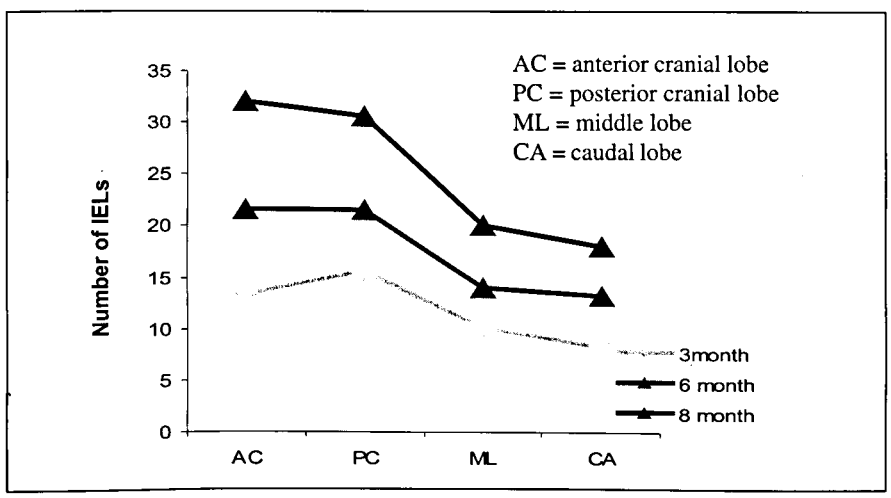

Figure 8: Number of IELs of lymphoid nodules in different lobes of lung at different ages of calves

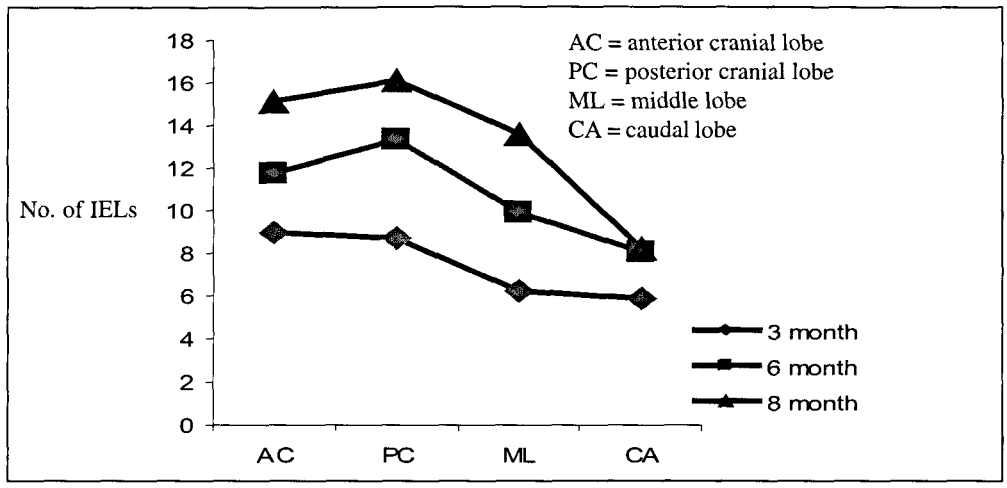

Figure 9: Number of IELs of lymphoid aggregates in different lobes of lung at different ages of calves

mammals (Bienenstock *et al.*, 1973; McDermott *et al.*, 1982; Anderson *et al.*, 1986a; Mair *et al.*, 1987; Huang *et al.*, 1990). The frequency of occurrence of BALT ranges from 100% in rabbit and rats, to 50% in guinea pigs, 33% in pig and 0% in cats (Pabst and Gehrke, 1990). In horse, BALT was absent immediately after birth, common in foals and less common in yearlings and 2-year-old animals (Blunden and Gower, 1999). In cattle, the mean number of BALT foci increases with age to the maximum at 18 months and then declines in adults (Anderson *et al.*, 1986a). The major species and age differences in BALT might be the reason for the marked differences in pulmonary immune responses between species and between individual of different ages (Pabst and Gehrke, 1990). The present study had found that the distribution of lymphoid nodules and lymphoid aggregates in the lung of calves varies between different ages and between different lobes of the same age. The number and size of lymphoid nodules and lymphoid aggregates increased with age. The findings are similar to the previous studies in cattle (Anderson *et al.*, 1986a), chicken and turkey (Fagerland and Arp, 1993), human (Tscherning *et al.*, 1995) and horse (Mair *et al.*, 1987; Horohov, 2004). However, the number of BALT declines in adult cattle (Anderson *et al.*, 1986a). Based on the current findings, it is suggested that the occurrence of BALT is influenced by age and location. Results of this study show that the lymphoid nodules and lymphoid aggregates are significantly higher ($P<0.05$) in the cranial lobes than other lobes.

Few aggregated lymphoid tissues and lymphoid nodules were found in the 3-month-old calves. Compared with most species (Bienenstock *et al.*, 1973a; Chen *et al.*, 1989; Mair *et al.*, 1987; Huang *et al.*, 1990), the intrapulmonary tract of 6- and 8- month-old calves possessed relatively more aggregated lymphoid tissues and lymphoid nodules and they were seen in bronchi, bronchioles and respiratory bronchioles. However, this finding is similar to the finding in equine and ovine (Mair *et al.*, 1987; Chen *et al.*, 1989). The number of IELs gradually increased with age and a regional variation in the number of IELs was found. Thus, the IELs may correlate with the region which firstly contacted with environmental air. This finding supports the observation by Horohov (2004). The number of IELs was considerably less in 3-month-old calves compared to that of 6- and 8-month-old calves. This finding suggests that the exposure to airborne antigens or formites stimulate the IELs. Boismenu and Havran (1994) and Goto *et al.* (2000) report that bronchial IELs may be stimulated by bronchial epithelial cell growth and may play important roles in airway mucosal immunity. According to Erie and Pabst (2000), IELs maintain non pathogenic allergy in the airway. Thus, an increase in the number of IELs may increase the mucosal immunity of the lung. According to morphometric analysis, it is concluded that the highest cellular organisations of BALT was observed in 8-month-old calves.

ACKNOWLEDGEMENT

This study was supported by the Ministry of Science, Technology and Innovation Malaysia IRPA Research Grant No 5484200. The authors wish to thank Mr. Mohd. Jamail Samad from the Histopathology Laboratory, Faculty of Veterinary Medicine, Universiti Putra Malaysia for technical support.

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