International Journal of Livestock Research, Vol. 11 (2) Feb' 2021
http://www.ijlr.org
eISSN: 2277-1964
NAAS Rating 2020 – 5.36

Original Research

Comparative Histomorphological Studies on the Lungs in Adult Pashmina, Bakerwali and Non-Descript Goats of Union Territories of Ladakh and Jammu & Kashmir**

Neelofar Nabi, Kamal Sarma* and Jonali Devi

Division of Veterinary Anatomy, Faculty of Veterinary Sciences and Animal Husbandry, SKUAST, Jammu, R. S. Pura, Jammu, Jammu and Kashmir, INDIA

**Part of PhD Thesis submitted to S. K. University of Agricultural Sciences & Technology, Jammu

*Corresponding Author: kamalsarma73@yahoo.com

How to cite this paper: Nabi, N., Sarma, K., & Devi, J. (2021). Comparative Histomorphological Studies on the Lungs in Adult Pashmina, Bakerwali and Non-Descript Goats of Union Territories of Ladakh and Jammu and Kashmir. *International Journal of Livestock Research*, 11(2), 155-161.
http://dx.doi.org/10.5455/ijlr.20201013073646

Received : Oct 12, 2020
Accepted : Jan 21, 2021
Published : Feb 28, 2021

Abstract

This study was conducted on the lungs of adult Pashmina, Bakerwali and non-descript goats (ten each) to demonstrate the histomorphological features. In the present study, the lungs of all the breeds of goat showed a well-developed visceral layer of the pulmonary pleura surrounding each lung lobe. The visceral pleura was composed of fibrous connective tissue with an outer mesothelial layer consisting of squamous cells. It consisted of mainly collagenous and bands of elastic fibres. The primary bronchioles of all the breeds of goat had less folded mucosa supported by a thin lamina propria. The secondary bronchioles had almost similar histological structure, except the reduced thickness of the bronchiolar wall, presence of non-ciliated Clara cells increased as compared to ciliated columnar cells and absence of goblet cell in the bronchiolar epithelium. The epithelium of the terminal bronchioles was composed of non-ciliated columnar or high cuboidal cells with relatively more numbers of Clara cells. The number of alveolar macrophages was more in the lungs of Bakerwali goat as compared to the other two breeds. Type-I pneumocytes were the most predominant cell type in the alveolar wall and inter alveolar septum which were simple squamous type of cells with agranular cytoplasm and prominent nuclei that were seen protruding into the lumen of the alveoli. The type-II pneumocytes were cuboidal cells with a foamy cytoplasm in all the goats under study.

Keywords: Bakerwali, Lungs, Non-descript Goats, Pashmina

Introduction

The respiratory system includes conducting portion, consisting of the nasal cavity, naso-pharynx, larynx, trachea and bronchi, while the respiratory portion, where gas exchange takes place consists of respiratory bronchioles, alveolar duct and alveoli. A major function of conducting portion is to condition the air before it enters the lungs, inspired air is cleansed, moistened and warmed (Banks, 1986). The histomorphology the respiratory tract might be influenced by the altitude. Hence, the present study has been designed to study the comparative histomorphological architecture of the lungs of Pashmina, Bakerwali and non-descript goats of J&K Union Territory as these three breeds are the normal habitants of different altitudes. Moreover, this is the first study of its kind in this aspect in available literature.

Materials and Methods

This study was conducted in the Division of Veterinary Anatomy, F. V. Sc. & A.H., R. S. Pura Jammu. The lung samples from adult Pashmina goats were collected from slaughter houses of U.T. of Ladakh. Similar organs of Bakerwali and non-descript goats were collected from slaughter houses in and around Jammu city. A total of ten samples from each goat breed were collected. Representative tissue samples from lungs of all the goats were preserved in 10% Neutral Buffered Formalin (NBF) solution (Luna, 1968) and these tissues were processed for paraffin block preparation by alcohol-benzene schedule (Luna, 1968), sections of 5 µm thickness were obtained from these blocks on clean glass slides and subjected to various histological methods as detailed here under.

Table 1: Different histomorphological stains used in the study

Purpose	Stain	Source
Histological stains		
Routine Histomorphology	Haematoxylin & Eosin	Luna (1968)
Collagen fibres	Mallory's Stain	Luna (1968)
Elastic fibres	Hart's Stain	Singh and Sulochana (1996)
Reticular fibres	Gomori's Stain	Singh and Sulochana (1997)
Nerve fibres	Bielschowsky's method	Singh and Sulochana (1997)

Results and Discussion

In the present study, the lungs of all the breeds of goat showed a well-developed visceral layer of the pulmonary pleura surrounding each lung lobe. The visceral pleurawas composed of fibrous connective tissue with an outer mesothelial layer consisting of squamous cells (Fig. 1). Dellmann and Brown (1987) observed well developed visceral layer of pleura in the lungs of the domestic animals, however, in carnivores, it was reported to be thin. Kalita (2014) also reported a thick and well-developed visceral layer of pleura in the lungs of Mizo pigs. It consisted of mainly collagenous fibres and bands of elastic fibres. Trabeculae originated from the visceral pleura which traversed inside the lung parenchyma as interlobar and interlobular septae. These were well developed and continued as interalveolar septae as also reported by Kalita (2014) and such lobulations accounted for distinct surface lobulations observed in the lungs of Mizo pig. These septae were composed of loose connective tissue along with elastic and sparse collagen fibres. Similar histological architecture was earlier reported by Kahwa and Purton (1996) in adult goats, Kalita and Bordoloi (2005) in yak and mithun and Dellmann and Brown (1987) in domestic animals.

Histomorphologically, different types bronchioles were observed in the lung tissue of all the breeds of goat viz. large bronchioles, terminal bronchioles and respiratory bronchioles. The respiratory bronchioles, alveolar ducts, alveolar sacs and alveoli formed the gas exchange components of the lungs. These findings were in corroboration to the earlier findings of Kalita and Bordoloi (2005) in yak, mithun and zebu, and Kalita (2014) in Mizo pigs. Also, similar airways and gas exchange components of the lung had been reported by Banks (1986) and Dellmann and Brown (1987) in domestic animals and Baba *et al.* (2016) in goats. The primary bronchioles of all the breeds of goat had less folded mucosa supported by a thin lamina propria. A thin layer of lamina muscularis mucosae surrounded each large bronchiole (Fig. 2). The large bronchioles were associated with blood vessels (arteries and veins). The mucosa of these bronchioles was composed of ciliated columnar epithelium with goblet cells. Among these cells,

clara cells, which were the non-ciliated short columnar cells distributed sparsely between the columnar cells. Similar histological architecture of the large bronchiole was also demonstrated in small animals (King, 1982) and in yak and mithun (Kalita and Bordoloi, 2005). The muscularis mucosae of these bronchioles showed smooth muscle fibres along with circularly oriented elastic and reticular fibres. In all the goat breeds under study, no cartilaginous plates or bronchial glands could be observed in the wall of the primary bronchiole. In contrary, Kalita and Bordoloi (2005) designated these primary bronchioles as large bronchioles and reported that few serous glands were present in their walls in mithun. The secondary bronchioles (Fig. 3) had almost similar histological structure, except the reduced thickness of the bronchiolar wall, presence of non-ciliated clara cells increased as compared to ciliated columnar cells and absence of goblet cell in the bronchiolar epithelium.

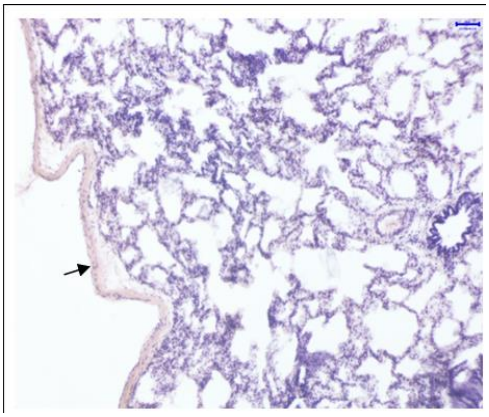

Figure 1: Photomicrograph showing the visceral pleura (arrow) of the lung of an adult non-descript goat. H&E, 100X.

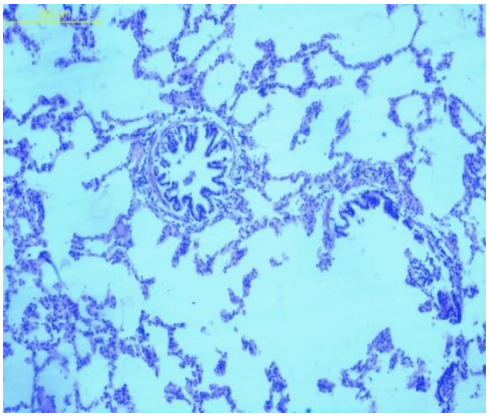

Figure 2: Photomicrographofa primary bronchiole showing lamina muscularis mucosae (arrow) of an adult non-descript goat. H&E, 100X.

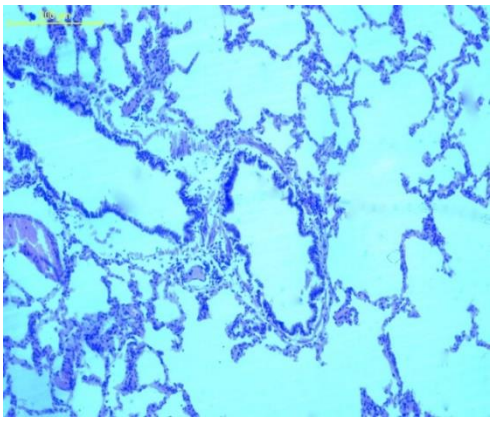

Figure 3: Photomicrograph showing a secondary bronchiole in the lung of an adult Pashmina goat. H&E, 100X.

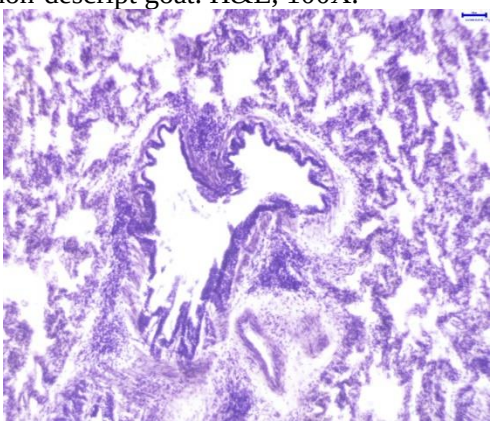

Figure 4: Photomicrograph showing a terminal bronchiole in the lung of an adult Pashmina goat. H&E, 100X.

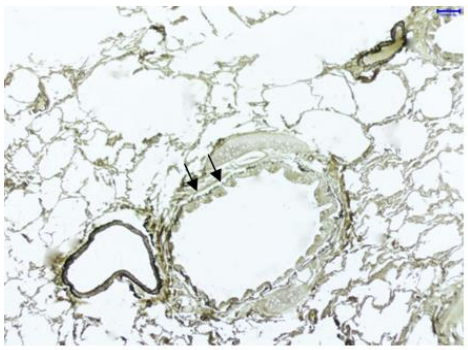

Figure 5: Photomicrograph showing reticular fibres (arrows) in the basement membrane of a terminal bronchus of an adult Bakerwali goat. Gomori's method, 400X

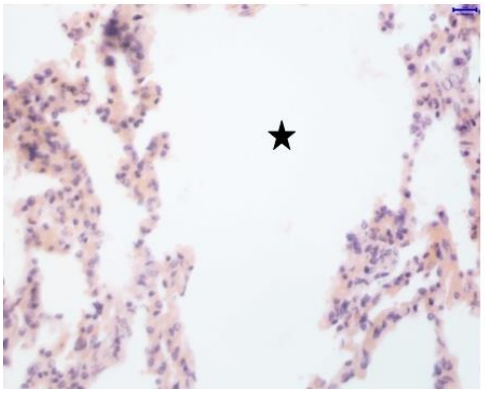

Figure 6: Photomicrograph showing alveolar sacs (star) in the lung of an adult non-descript goat. H&E, 400X.

Similar observations in regard to the histological structure of the secondary bronchioles had been reported earlier in domestic animals (Dellmann and Brown, 1987), Yak and mithun (Kalita and Bordoloi, 2005) and in Mizo pig (Kalita, 2014). It was noticed in the present study that the secondary bronchioles gave rise to 3-4 terminal bronchioles in case of Pashmina goat and the value in regard to Bakerwali and non-descript goats was 2-3 numbers. Comparable number of terminal bronchioles was also reported to be emerged from the individual secondary bronchioles in goat (Kahwa *et al.,* 1997). Also, Kalita and Bordoloi (2005) found 2-3 numbers of terminal bronchioles emerging from each large bronchiole in yak and Mithun. The epithelium of the terminal bronchioles (Fig. 4) was composed of non-ciliated columnar or high cuboidal cells resting on a basement membrane composed of fine reticular fibres (Fig. 5) with relatively more numbers of clara cells. This was in contrary to the findings of Write *et al.* (1983) and Iovannitti *et al.* (1985) in dog and calf, respectively, who reported that the epithelium of the terminal bronchioles was principally composed of ciliated columnar cells. Again, presence of mostly a population of ciliated columnar cells in the epithelium of the terminal bronchioles of the lungs in horse and sheep was also observed by Pirie *et al.* (1990) and Bouljihad and Leipold (1994), respectively.

However, in corroboration to the present findings, Kalita and Bordoloi (2005) and Kalita (2014) reported that the epithelium of the terminal bronchioles was formed mainly by non-ciliated columnar or tall cuboidal cells in Mizo pig and Yak, respectively. The increased number of clara cells as observed in the present study was in agreement with the report of Kalita and Bhattacharya (2004) in Mithun and zebu, and dog and cat (Dellmann and Eurel, 1998). The basement membrane of the terminal bronchioles was indistinct as also reported in 12-month-old goats (Suman *et al.,* 2005). The lumen of the terminal bronchiole was spherical in outline. The clara cells of the terminal bronchiolar epithelium were low cuboidal cells having varying degree of cytoplasmic granules with a prominent centrally placed nucleus as also reported in sheep (Mariassy and Plopper (1983) and in Mithun and yak (Kalita and Bordoloi, 2005). The lamina propria consisted of loose connective tissue with few lymphocytes and elastic fibres. The lamina muscularis mucosaa was composed of 1-2 layers of circularly oriented smooth muscle cells. Dellmann and Brown (1987) however, reported that the terminal bronchioles of the domestic animals had a single layer of smooth muscle cell which was circularly oriented. A thin layer of loose connective tissue was observed surrounding this muscle layer. In the present study it was seen that 6-8 generations of respiratory bronchioles were originated from individual terminal bronchioles in Pashmina goat and 4-6 generations of the same were originated in Bakerwali and non-descript goats of Jammu region. Such generations of respiratory bronchioles with their origin from the terminal bronchioles as observed in the present study could be comparable with the earlier reports of Kalita and Bordoloi (2005), who recorded that the distal terminal bronchioles gave off 5-8 generations of respiratory bronchioles in mithun and 5-6 generations of respiratory bronchioles in zebu. On the other hand, lesser number (2-3) of the same had been reported in canines and felines (Banks, 1986).

The respiratory bronchioles originated from the distal terminal bronchioles in all the goat breeds under study. At one end, the contour of the lumen of the respiratory bronchiole was rounded and the other side, the epithelium was interrupted to be continued as alveolar ducts. The respiratory bronchioles were lined by a single layer of cuboidal epithelium without goblet cells. A distinct basement membrane was lacking and the cells rested on a composite network of smooth muscle cells, collagen, reticular and few elastic fibres. Solitary aggregations lymphoid cells were also detected in the wall of the respiratory bronchioles. Similar observations were also narrated in large ruminants (Dellmann and Brown, 1987) and in year old goats (Suman et al., 2005). The lumen of the respiratory bronchioles of the Pashmina goat apparently looked wider than the other two breeds of goat. Clara cells were seen populating between the cuboidal cells which had got protruding nuclei into the bronchiolar lumen. Population of non-ciliated clara cells was also reported in the respiratory epithelium of domestic animals (Banks, 1983), sheep (Bouljihad and Leipold, 1994), goat (Kahwa *et al.,* 1997) and mithun, yak and zebu (Kalita and Bordoloi, 2005). Banks (1986) opined that the proteolytic and mucolytic enzymes were secreted by the clara cells which helped to keep the airways open for optimum respiratory processes. The cuboidal epithelial layer of the respiratory bronchioles had several interruptions by the alveoli that sprang out from the wall of the respiratory bronchioles. Well-developed respiratory bronchioles were also observed in the lungs of Mizo pigs by Kalita (2014). In contrary, Bal and Ghoshal (1988) reported that the small laboratory animals like rat, mouse, hamster, guinea pig, gerbil and rabbit lacked respiratory bronchioles. More generations (6-8) of well-developed respiratory bronchioles as recorded in the present study in Pashmina goat might be to facilitate the respiratory ability in this breed of goat to thrive them in their habitat with higher altitudes having much lower atmospheric oxygen pressure like U.T. of Ladakh region as also reported in Mizo pigs (Kalita, 2014).

The alveolar ducts were observed as tubular structures that extended from the respiratory bronchioles into which many alveoli open on both the sides. These followed a tortuous course with branching. It was observed that the alveolar ducts in all the breeds of goat were lined by simple low cuboidal epithelium. Some alveolar ducts were also noticed lined with simple squamous cells. Scant connective tissue with collagen and elastic fibres and occasional smooth muscle cells supported the epithelium. Occurrence of smooth muscle cells was seen mostly at the origination site of the alveoli from these alveolar ducts. Similar histological features of the alveolar ducts were also reported in domestic animals (Dellmann and Brown, 1987; Bacha and Bacha, 2000), in Mizo pigs (Kalita, 2005) and in human (Ross and Pawlina, 2010). The alveolar sacs or the atria were the large sized sac like cavities into which adjacent alveoli opened (Fig. 6). They were lined by simple squamous or simple cuboidal epithelium.

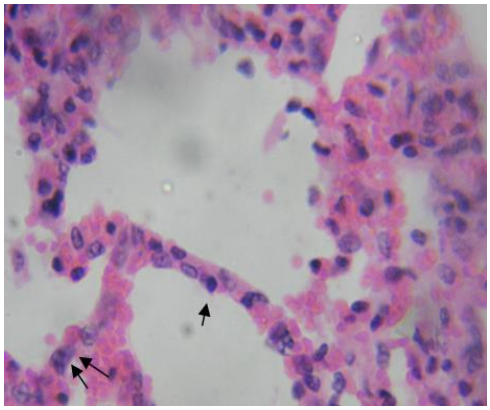

Figure 7: Photomicrograph showing pneumocytes-I (1) and pneumocytes-II (2) in the lung of an adult Pashmina goat. H&E, 400X.

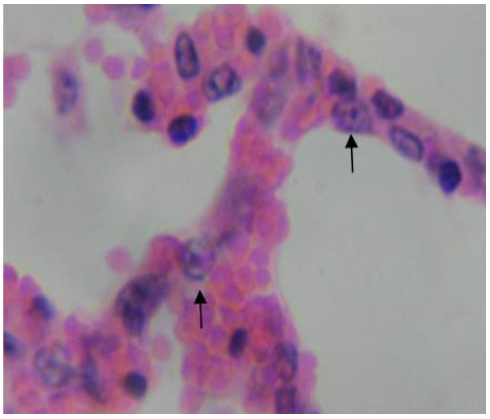

Figure 8: Photomicrograph showing pneumocytes-II (arrow) in the lung of an adult Pashmina goat. H&E, 1000X.

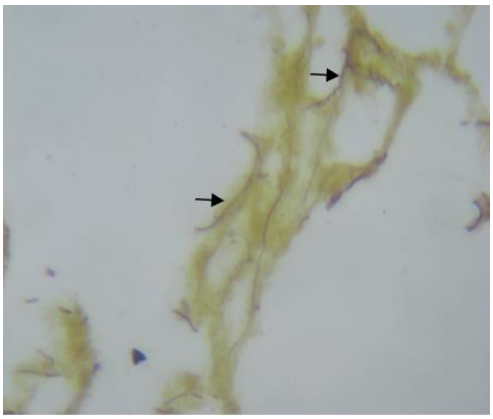

Figure 9: Photomicrograph showing elastic fibres in the alveolar wall (arrows) in the lung of an adult Pashmina. Hart's stain, 400X.

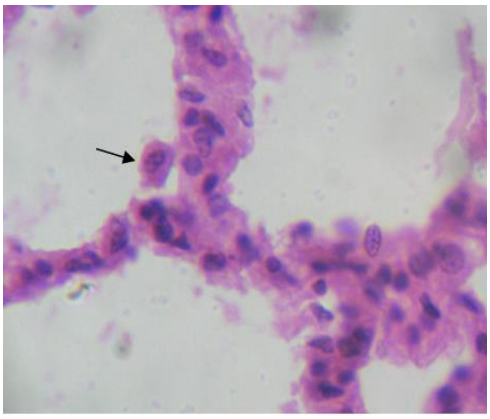

Figure 10: Photomicrograph showing an alveolar macrophage (arrow) in the lung of an adult Pashmina goat. H&E, 1000X.

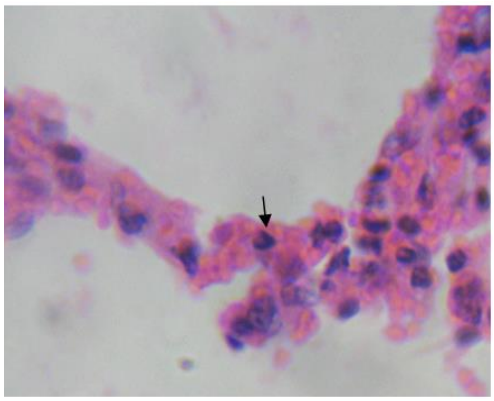

Figure 11: Photomicrograph showing an alveolar macrophage with indented nucleus (arrow) in the lung of an adult Pashmina goat. H&E, 1000X.

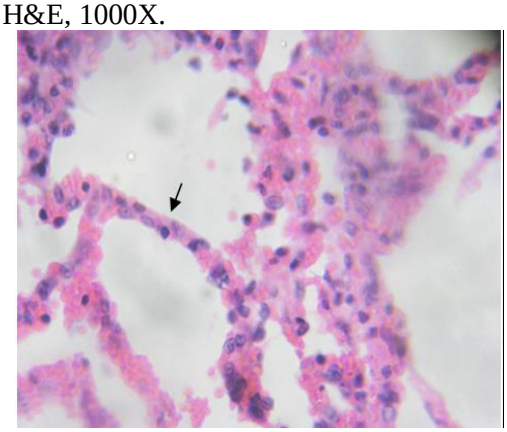

Figure 12: Photomicrograph showing alveolar wall (arrow) in the lung of an adult non-descript goat. H&E, 400X.

The alveoli were the terminal segments of the respiratory passages which formed the basic structural and functional unit for gas exchange in the lung parenchyma (Cormack, 1987). In the present study, the alveolar walls were lined mainly by two cell types- the agranular Type-I pneumocytes and granular epithelial cells which included the Type-II pneumocytes and alveolar macrophages or dust cells (Fig. 7). In contrary to the present findings, Kahwa and Purton (1996) reported that the alveoli were lined by only simple squamous type of epithelium in goat. However, later on, Kahwa *et al.* (1997) observed two cell types viz. Type-I and Type-II pneumocytes in the alveolar wall of the same species, which corroborated to our present study. In the present study, the Type-I pneumocytes were the most predominant cell type in the alveolar wall and inter alveolar septum. These cells were reported to be chiefly related with maintenance of interface between the blood and air for allowing an optimum gaseous exchange in domestic animals (Banks, 1993). These were simple squamous type of cells with agranular cytoplasm and prominent nuclei that were seen protruding into the lumen of the alveoli. The less abundant type-II pneumocytes were observed as simple cuboidal type of cells which possessed large prominent nuclei (Fig. 8). Banks (1993) reported that the type-II pneumocytes was responsible for secretion of pulmonary surfactant, which was a detergent like substance and helped to reduce the surface tension of the lung alveoli and also prevented alveolar collapse during expiration of air. Similar notes on the significance of the type-II pneumocytes were also opined by Craigmyle (1986) and Plopper and Adams (1993) in domestic animals. The type-II pneumocytes had a foamy cytoplasm in all the goats under study and they protruded into the alveolar lumen. Another cell type, called alveolar brush cell had been reported in rats (Banks, 1986). However, they could not be detected in the present study in the lungs of goat. These epithelial cells of the alveolar wall were supported by a very thin network of connective tissue comprising fine collagen, elastic and reticular fibres (Fig. 9).

In the present study, the alveolar macrophages or the dust cells were found either in the alveolar wall or in the lumen of the alveoli (Fig. 10). They were large cells with granular cytoplasm occasionally with indented nuclei (Fig. 11). Presence of alveolar macrophages had been also reported earlier in domestic animals (Dellmann and Brown, 1987; Banks, 1986; Plopper and adams, 1993 and Bacha and Bacha, 2000), cattle (Iovannitti *et al.*, 1985), in goat (Baba and Choudhury, 2008) and human (Cormack, 1987 and Ross and Pawlina, 2010). It was reported that the alveolar lumina of goat possessed few macrophages (Bhattacharya *et al.*, 1996). But, Bouljihad and Leipold (1994) reported very scant pulmonary alveolar macrophages in sheep. However, Iovannitti *et al.* (1985) opined that the macrophages were commonly found in the alveolar walls in the lungs of calf and adult cattle. In our study, it was observed that the number of alveolar macrophages was more in the lungs of Bakerwali goat as compared to the other two breeds, which had also been confirmed by using immunohistochemical methods. Such higher population of macrophages or dust cells present in the lungs of Bakerwali goat might facilitate them extra protection from the air borne antigens as it is typically a migratory type of breed. Occurrence of high population of pulmonary macrophages had also been reported in Mizo pigs which were known for better resistant to respiratory diseases (Kalita, 2014). Plopper and Adams (1993) also opined that the alveolar macrophages were considered to be associated with the macrophage system of the body that had a protective role by dint of their defense system. In the present study, the interalveolar septae were present between the adjacent alveoli (Fig. 12). These were covered on the both the sides by alveolar epithelium with a centrally placed connective tissue core consisting of collagen, elastic and reticular fibres. There was an extensive network of blood vessels present in this connective tissue core. These findings were in corroboration with the same reported in domestic animals (Dellmann and Brown, 1987 and Banks, 1986), goats (Kahwa and Purton, 1997 and Baba and Choudhury, 2008) and Mizo pigs (Kalita, 2014).

Conclusion

The primary, secondary and terminal bronchi had similar histological structures in all the breed of goats. The histoarchitecture of all the generations of bronchioles were similar. The Type-I pneumocytes were the most predominant cell type in the alveolar wall and inter alveolar septum. No variations due to altitude was found in regard to histomorphology of the lungs in various breeds of goat under study.

Conflict of Interests

There is no conflict of interest.

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