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Light and Ultrastructural Studies on the Lungs of Mizoram Red Serow (*Capricornis rubidus*)

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ABSTRACT

Background: The present study was aimed for the promotion and advancement of the anatomical knowledge at the light microscopic and electron microscopic level in red serow (*Capricornis rubidus*).

Methods: The present work was carried out at the department of Veterinary Pathology, Department of Veterinary Anatomy and Histology, College of Veterinary Sciences and Animal Husbandry, Central Agricultural University, Selesih, Aizawl, Mizoram and Sophisticated Analytical Instrument Facility (SAIF), All India Institute of Medical Sciences, New Delhi. Three Lungs samples were collected from three apparently healthy red serow of either sex. Thereafter tissue samples were collected as such and were preserved in neutral buffer formalin (NBF) and in Karnovsky's fixative for routine histology and transmission electron microscopic examination, respectively.

Result: The bronchi were lined by pseudostratified ciliated columnar epithelium. Goblet cells, basal cells and migratory cells were identified in different order of bronchus but the number of goblet cells decreased from primary to tertiary. Two orders of bronchioles were identified in Red Serow. The shape of the bronchioles was mostly round to elliptical on cross sectional view. The alveolar ducts were found as tubular structures surrounded by alveoli and usually followed a long tortuous course and gave off several branches. The walls of the alveolar ducts consisted of open sides of alveoli and the terminations of the inter alveolar septa which separated the alveoli. Two cell types, the ciliated and the non-ciliated bronchiolar epithelial (Clara) cell, formed the major components of the cell population, with the mucus-producing cell being observed only occasionally. The cells rested on a prominent basal lamina and the whole epithelium was thrown into folds. Ciliated cells were observed in the terminal bronchioles and as far distally as the respiratory bronchioles and were seen to vary both in number and height. Non-ciliated bronchiolar epithelial (Clara) cells were observed in the terminal bronchioles and all the way into the respiratory bronchioles, where they were in the majority.

Key words: Histology, Lungs, Red serow, Ultrastructure.

INTRODUCTION

Capricornis rubidus, commonly known as the red serow, represents a distinctive member of the *Caprinae* subfamily distributed across limited regions in northern Myanmar and southern Bangladesh. Historically, some authors have regarded it as a subspecies of *Capricornis sumatraensis*, yet contemporary taxonomic assessments recognize it as a separate entity. Within the Indian subcontinent, the red serow's presence is predominantly recognized in the hilly terrains south of the Brahmaputra River in the northeast, although authoritative records primarily affirm populations in Myanmar as per IUCN (2008) documentation. The species is currently assessed as Near Threatened on the IUCN Red List, a status that accentuates the urgency for comprehensive baseline studies covering its anatomical and physiological characteristics to bolster future conservation initiatives.

Despite its recognized ecological role and conservation priority, the anatomical and histological attributes of the red serow's pulmonary system remain poorly characterized. Among ruminants, pulmonary architecture is intimately tied to physiological adaptations for survival in diverse environments, including variable altitudes and activity demands.

Detailed examination of pulmonary architecture in domestic goats (*Capra hircus*) and related *Caprinae* has yielded valuable anatomical and ultrastructural insights. For example, Baba and Choudhary (2008) reported the presence of both Type I and Type II pneumocytes in goat alveoli, with an average alveolar diameter of about 45 µm. Developmental investigations by Gupta and Jain (2007) revealed age-dependent changes in bronchiolar histochemistry, notably the emergence of PAS-positive bronchiolar epithelial cells during the first year of life. Ultrastructural analyses conducted by Kumar and Kumar (2024) using transmission

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electron microscopy identified a delicate blood-air barrier comprised of Type I and II pneumocytes, a basement membrane and capillary endothelium. Comparative studies across various goat breeds highlight notable morphologic differences, including variable pleural thickness, distinctive features in the bronchiolar lining epithelium and diversity in the presence and distribution of Clara cells and alveolar macrophages (Nabi and Devi, 2020; Yousif, 2021). While some anatomical data exist for wild Caprinae, such as the Japanese serow (*Capricornis crispus*; Nakakuki, 1986), no equivalent light or electron microscopy-based research describing lung parenchyma has been published for the red serow (*C. rubidus*). This lack of species-specific data underscores a critical gap in anatomical knowledge for this near threatened species.

To address this deficiency, the present study was designed with the following objectives: (1) to delineate the detailed histological features of the red serow lung tissue utilizing light microscopy and (2) to describe the ultrastructural properties of bronchiolar and alveolar components through transmission electron microscopy. Through integration of these methodologies, this research aims to establish a vital anatomical reference point for C. rubidus, thereby enabling more informed comparative, physiological and conservation-oriented studies of this rare ungulate.

MATERIALS AND METHODS

Three lung samples were collected from three apparently healthy adult red serows (*Capricornis rubidus*), aged approximately 18 years, of either sex, during post-mortem examinations conducted at the Veterinary Hospital, Lungdai, Mizoram. The animals had died naturally of senescence (old age) and were examined within 45 minutes post-mortem. Approximately eight tissue blocks were collected from the lung parenchyma of each specimen for histological and ultrastructural studies. Tissue collection was performed with the consent of the attending veterinarian and in accordance with the guidelines of the State Forest and Wildlife Department, Mizoram. Only small tissue fragments were obtained post-mortem; no animals were sacrificed for this research.

Histology methods

Fresh lung tissues were fixed in 10% neutral buffered formalin (NBF) for 24-48 hours. Fixed tissues were then processed using the standard paraffin-embedding method, consisting of:

- **Dehydration:** Graded ethanol (70%, 80%, 90% and absolute alcohol; 1 h each).
- **Clearing:** Cedarwood oil for 30 min.
- **Embedding:** Paraffin wax (melting point 58-60°C) using a Leica RM 2245 semi-motorized rotary microtome.
- Sections of 6 µm thickness were stained with haematoxylin and eosin (H and E) following Mayer's method (1893) to examine the normal tissue architecture. For each sample, five representative sections were studied under light microscopy using a systematic random sampling strategy to minimize bias. Slides were examined with a Leica DM2500 light microscope at 10×, 40× and 100× (oil immersion) magnifications. Digital photomicrographs were captured with an in-built Leica DFC450 C camera and images were analyzed using ImageJ software (NIH, USA).

Transmission electron microscopy (TEM)

For ultrastructural analysis, lung tissues were processed following standard TEM protocols (Karnovsky, 1965) with minor modifications.

i. **Fixation:** Samples were fixed in Karnovsky's fixative (2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.2) for 2-4 h at 4°C, washed three times (15 min each) in 0.1 M buffer and transported to the sophisticated analytical instrument facility (SAIF), AIIMS, New Delhi, for further processing because local TEM facilities were unavailable. During transport, tissues were maintained in buffer at 4°C and processed within 24 h.

ii. **Washing:** Fixed tissues were washed three times (15 min each) in 0.1 M phosphate-buffered saline (PBS, pH 7.4) at 4°C.

iii. **Post-fixation:** Samples were post-fixed in 1% osmium tetroxide for 2 h at 4°C, followed by three PBS washes.

iv. **Dehydration:** Graded acetone (30%, 50%, 70%, 80%, 90% and 100%) for 30 min each at 4°C, then dry acetone for 30 min at room temperature.

v. **Clearing:** Toluene I and II for 30 min each.

vi. **Infiltration:** Sequential infiltration in embedding-medium: Toluene mixtures (3:1, 2:1, 1:1) for 12 h each; final stage under vacuum.

Embedding medium composition

- Araldite CY212-10 ml.
- DDSA (Dodecenyl Succinic Anhydride)-10 ml.
- DMP-30 (2,4,6-Tris(dimethylaminomethyl phenol)-0.4 ml.
- Plasticizer (Dibutyl Phthalate)- 1.0 ml.

vii. **Embedding and polymerization:** Infiltrated tissues were embedded in pure embedding medium using gelatin capsules and polymerized at 50°C for 24 h, then 60°C for 48 h.

viii. **Ultrathin sectioning:** Silver-grey sections (70-80 nm) were cut using a Leica Ultracut UCT ultramicrotome with a diamond knife and mounted on copper grids.

ix. **Negative staining:** Sections were stained with 2% uranyl acetate for 15 min and lead citrate for 10 min (Reynolds, 1963).

x. **Microscopy:** Grids were examined under a JEOL JEM-2100 transmission electron microscope (Japan) operating at 80 kV and images were captured with the built-in high-resolution digital camera at 5,000× -30,000× magnification.

Data recording and interpretation

Preliminary interpretations were made during TEM observation and detailed analyses were performed from

photomicrographs. The study focused on qualitative histological and ultrastructural description; therefore, no quantitative morphometric or statistical analysis was undertaken. However, descriptive measurements of cell and organelle dimensions were recorded where relevant.

RESULTS AND DISCUSSION

Histological examination

The bronchi of the red serow were lined by pseudostratified ciliated columnar epithelium (Fig 1). Goblet cells, basal cells and migratory cells were identified in different orders of bronchi, but the number of goblet cells decreased from the primary to the tertiary bronchi. The height of the epithelium also decreased gradually from primary to tertiary bronchi. Similarly, the thickness of the propria and submucosa progressively decreased.

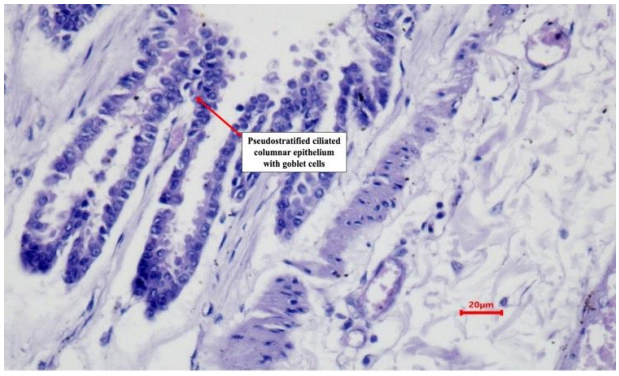
The propria and submucosa contained mixed submucosal glands and the frequency of these bronchial glands gradually diminished in the red serow. Hyaline cartilage appeared as regular plate-like structures in the primary bronchus, while from the secondary to tertiary bronchus, irregular cartilage plates and smooth muscle fibers were interspersed along the luminal side of the plates. The muscular components were arranged in circular fascicles, with cartilage thickness and width diminishing distally, whereas the muscular component became more abundant from primary to tertiary bronchi (Fig 2).

The adventitial connective tissue was loosely arranged, containing abundant collagen fibers and few elastic fibers, with fiber orientation perpendicular to the long axis of the airway. The number of mucosal folds increased progressively from primary to tertiary bronchi.

Under special staining, abundant elastic fibers were observed in the propria and submucosa, particularly around the cartilaginous plates. Two orders of bronchioles were identified in this species (Fig 3). The bronchioles were round to elliptical in cross-section. The first-order bronchioles were lined by simple ciliated columnar epithelium and the second-order bronchioles by simple cuboidal epithelium. Clara cells were also identified. Glands and cartilaginous plates were absent in the bronchioles and the propria contained a thin layer of loose connective tissue with smooth muscle fibers arranged in circular and oblique manners (Fig 3).

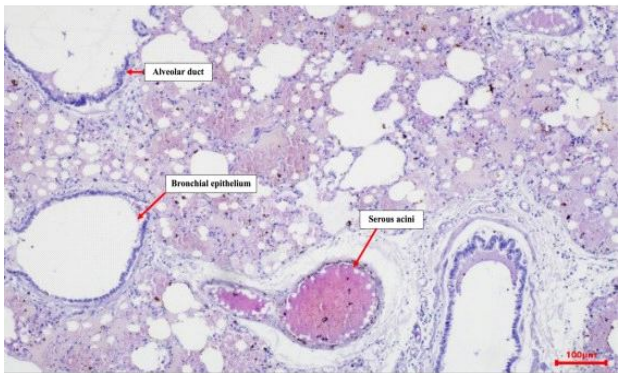
The adventitia was composed of loose connective tissue with abundant elastic fibers. Small dome-shaped lymphoid follicles were found close to some bronchioles (Fig 3). Respiratory bronchioles were infrequently observed as outpocketings of the bronchiolar wall (Fig 4), lined by simple cuboidal epithelium, occasionally interrupted by alveolar epithelium. The lamina propria was indistinct and smooth muscle fibers were arranged in fascicles beneath the epithelium, with alveolar openings between the fascicles.

Alveolar ducts divided into numerous small sacs (saccules) lined by simple squamous epithelium (Fig 5). Type I pneumocytes predominated in the alveolar lining; their nuclei projected into the lumen and the basal lamina was continuous. Type II granular epithelial cells appeared cuboidal with centrally placed nuclei and occasional



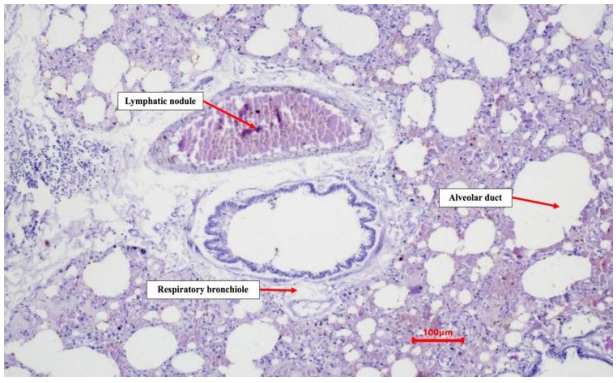
H and E, 40x.

Fig 1: Photograph showing bronchi of red serow, lined by pseudostratified columnar epithelium.



Alveolus is seen within the epithelium. H and E, 40x.

Fig 2: Photograph showing lungs parenchyma of red serow.



One respiratory bronchiole and alveolar duct is also present. H and E, 40x.

Fig 3: Photograph showing one lymphoid follicle in the lung parenchyma of red serow.

lamellar bodies. A few free alveolar macrophages were present within the alveolar lumen. The interalveolar septum contained collagen, elastic and reticular fibers, as well as fibrocytes, phagocytes and macrophages. The outermost lung covering consisted of collagen and elastic fibers.

Transmission electron microscopy (TEM)

Two main cell types-ciliated and nonciliated bronchiolar epithelial (Clara) cells-formed the major epithelial population (Fig 6), while mucus-producing cells were observed only occasionally. The epithelium of respiratory bronchioles was mainly simple cuboidal, occasionally interrupted by simple squamous areas.

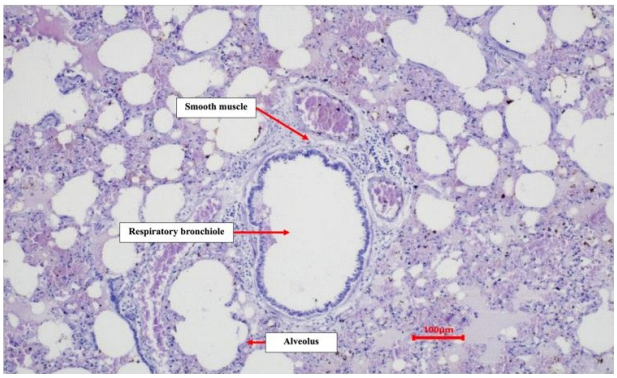
The alveolar membrane comprised a simple squamous epithelial lining, a central capillary and variable connective tissue. Alveolar Type I and Type II cells were present (Fig 7).

Ciliated cells

Ciliated cells were present from the terminal to the respiratory bronchioles. They varied in height and number, being columnar in terminal bronchioles and cuboidal in respiratory bronchioles. Each cell had cilia and microvilli at the luminal surface, with cilia anchored by basal bodies. The cytoplasm was electron-lucent, containing an oval basal nucleus, Golgi apparatus, mitochondria and smooth endoplasmic reticulum near the apical region (Fig 8). Developing ciliated cells displayed microvilli and basal bodies, indicating stages of ciliogenesis.

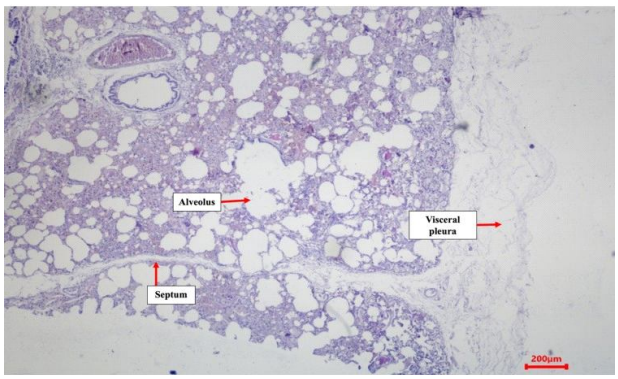
Nonciliated bronchiolar (Clara) cells

Clara cells were columnar to cuboidal, often with apical protuberances and short microvilli (Fig 6). The cytoplasm was electron-dense, containing smooth endoplasmic reticulum, elliptical mitochondria and electron-dense secretory granules. The nucleus was centrally placed. Tight junctions connected adjacent cells and interdigitations occurred basally.



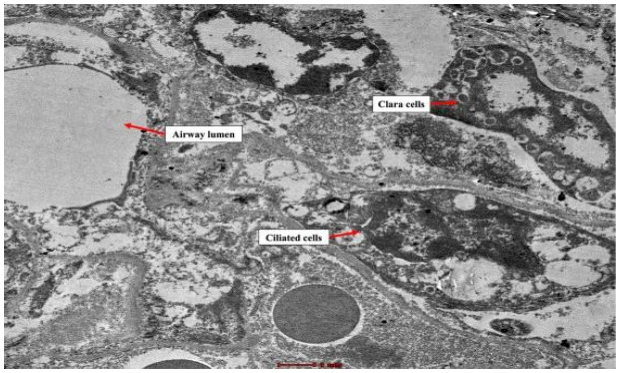
H and E, 40x.

Fig 4: Photograph showing respiratory bronchiole in the lungs of Red Serow: Alveolus and smooth muscle is also seen the lung parenchyma.



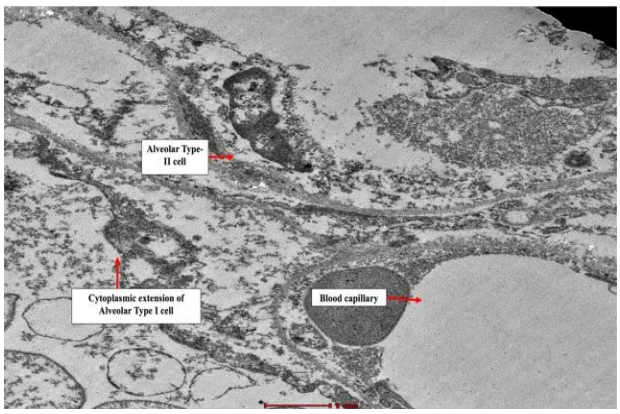
Incomplete septa extend inward from the visceral pleura. Alveolus is seen within the epithelium. H and E, 40x.

Fig 5: Photograph showing visceral pleura of red serow.



Simple columnar epithelium composed of ciliated cells and nonciliated bronchiolar epithelial (Clara) cells resting on a prominent basal lamina (arrow). Airway lumen. TEM x 5,400

Fig 6: Photomicrograph showing TEM image of Terminal bronchiole.



Note the protruding alveolar Type II cell, blood capillary and cytoplasmic extensions of alveolar Type I cell carrying a few surface microvilli. TEM x 5,400.

Fig 7: Photomicrograph showing TEM image of alveolar membrane.

Mucous-producing cells

Occasionally observed in terminal bronchioles, these cuboidal cells had short apical microvilli, a basal nucleus and numerous heterogeneous granules in the supranuclear region (Fig 9). The cytoplasm contained rough and smooth endoplasmic reticulum.

Alveolar type I cells

These flattened cells had oval nuclei, long cytoplasmic extensions and few organelles, though pinocytotic vesicles were present. Short microvillus-like projections occurred on the luminal surface (Fig 6).

Alveolar type II cells

These cuboidal cells bulged into the alveolar lumen (Fig 7). They contained lamellated inclusion bodies, electron-dense cytoplasm, numerous mitochondria, rough endoplasmic reticulum, Golgi apparatus and lipid vacuoles of variable size. The nucleus was large and central, often with a prominent nucleolus.

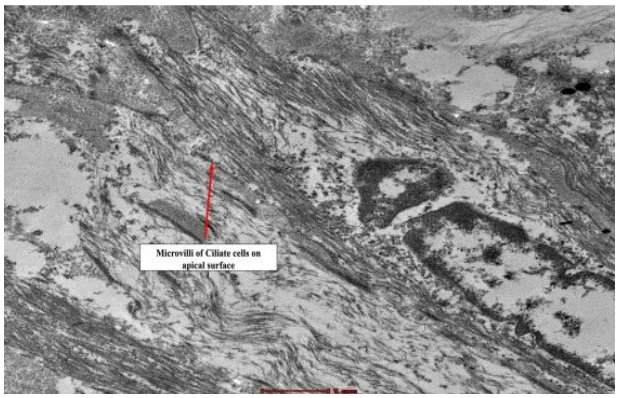
Alveolar septum and macrophages

The alveolar septa contained attenuated capillary endothelial cells with deep cytoplasmic invaginations (Fig 7). Connective tissue between epithelial and endothelial basal laminae contained collagen fibers, fibroblasts and mast cells. Where connective tissue was sparse, basal laminae fused.

Alveolar macrophages were free within the alveolar spaces (Fig 10), with irregular nuclei, smooth and rough endoplasmic reticulum, numerous mitochondria and large vesicles and vacuoles containing osmiophilic material.

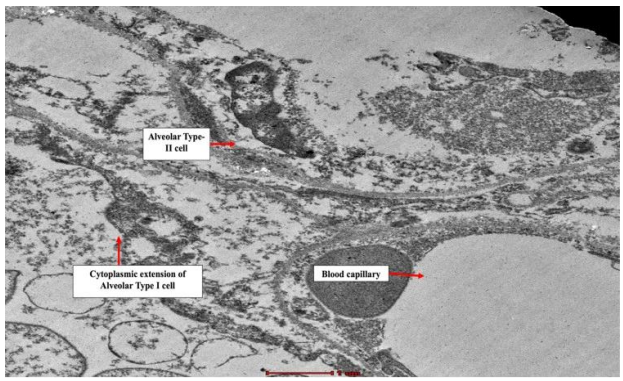
The histological and ultrastructural organization of the red serow lung closely resembled that of other ruminants, particularly goats and cattle. Dellmann and Brown (2006) described the bronchial epithelium of goats as pseudostratified ciliated columnar with numerous goblet cells-consistent with the present observations. Similarly, the gradual reduction of glandular and cartilaginous components toward the distal airways supports earlier reports by Banks (1993), Khyalia *et al.* (2019) and Bacha (1990). This reduction likely facilitates decreased airway rigidity and enhanced flexibility, enabling efficient airflow distribution in the smaller bronchioles. The observed increase in mucosal folds and elastic fibres in smaller bronchi corresponds to the adaptive requirement for elasticity and the maintenance of airway patency during respiration. The absence or reduction of bronchial glands, as noted by Dellmann and Brown (2006) in goats and reflected in the red serow, may be associated with minimizing mucus accumulation, thus optimizing airflow in drier montane environments. Nabi *et al.* (2021) conducted comparative micrometrical studies on the lungs of different goat breeds and reported that larger alveolar spaces and thinner inter-alveolar septa enhance gaseous exchange efficiency in animals adapted to high-altitude environments; a finding that closely parallels the structural refinements observed in the red serow lung.

The organization of the bronchiolar and alveolar regions corresponded closely with that of domestic



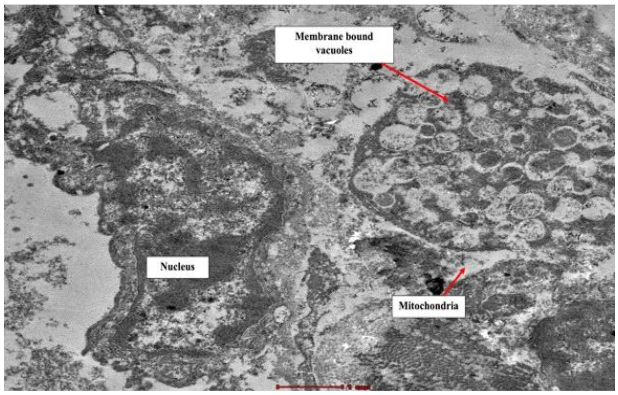
Part of the nucleus is visible, TEM x 20,000.

Fig 8: Photomicrograph showing TEM image of Ciliated cell. Note cilium and numerous microvilli on luminal surface.



Profiles of rough endoplasmic reticulum are distributed throughout the cytoplasm. TEM x 10,000.

Fig 9: Photomicrograph showing TEM image of Numerous heterogeneous secretory granules.



Note the large nucleus, ellipsoid mitochondria and large membrane-bound vacuoles, TEM x 20,000.

Fig 10: Photomicrograph showing TEM image of alveolar macrophage.

ruminants (Banks, 1993; Bacha, 1990). The presence of dome-shaped lymphoid follicles near small bronchioles, as similarly observed in buffalo by Yadav *et al.* (2005), suggests the presence of localized bronchus-associated lymphoid tissue (BALT) that contributes to pulmonary immune defense. The alveolar ducts and sacs exhibited interwoven collagen and elastin networks, in agreement with the observations of Baba and Choudhary (2008) and the biomechanical interpretations of Mercer and Crapo (1990), who emphasized the role of these fibers in maintaining alveolar integrity and supporting efficient parenchymal recoil during ventilation. Such structural arrangements in the red serow may thus reflect a functional adaptation that enhances the lung's mechanical efficiency in sustaining prolonged activity across steep, oxygen-variable terrains.

The histological and ultrastructural organization of the red serow lung closely resembled that of other ruminants, particularly goats and cattle, with normal architecture preserved throughout the bronchiolar and alveolar regions. The absence of pathological alterations such as septal thickening, emphysema, or inflammatory infiltration suggests that the species maintains a structurally intact respiratory system suited for efficient gaseous exchange in its mountainous environment. In contrast, Behera *et al.* (2025) documented frequent pulmonary lesions in slaughtered bovines-including interstitial pneumonia, emphysema, and broken or distended alveoli-and emphasized that "pneumonia is important in animals because of extreme weather conditions during dry season and verminous pneumonia in rainy season"; however, no such environmentally associated lesions were observed in the red serow, whose lungs retained normal architecture without evidence of disease-related compromise.

Ultrastructural features further revealed a high degree of conservation across mammalian taxa. The ciliated and Clara cells of the red serow displayed morphological characteristics comparable to those of dogs, pigs and horses (Majid, 1986; Baskerville, 1970; Pirie, 1990). The absence of glycogen granules in Clara cells, similar to that reported in guinea pigs and rodents (Plopper *et al.,* 1980a) but differing from oxen and cats (Plopper *et al.,* 1980b), may suggest species-specific metabolic modulation of these non-ciliated secretory cells. Such a feature might relate to the red serow's adaptation to variable oxygen availability, where reduced glycogen stores could correspond to a lower reliance on anaerobic metabolism in airway epithelium. The alveolar Type I and Type II pneumocytes also exhibited characteristic features described in goats (Atwal and Sweeny, 1971) and in Guinea Pigs (Rajathi, 2024) with lamellated bodies in Type II cells indicating active surfactant production and pinocytotic vesicles in Type I cells confirming their role in gas exchange. Together, these cells ensure alveolar stability and efficient oxygen diffusion-critical for mammals inhabiting high-altitude ecosystems.

The presence of numerous alveolar macrophages resembling those reported in other ruminants (Epling, 1964; Pirie, 1990) underscores the conserved nature of pulmonary defense mechanisms across species. Their abundance in the red serow may indicate enhanced immunological vigilance, essential in wild habitats where exposure to environmental particulates and pathogens is higher. Integrating both histological and ultrastructural observations, the present findings collectively illustrate a coherent structure-function relationship: The elastic, highly vascularized and surfactant-active lung of the red serow is well adapted for efficient gaseous exchange under demanding mountainous conditions.

From an evolutionary perspective, the lung of the red serow retains the general Caprinae plan but exhibits subtle adaptive refinements that support its ecological specialization. The combination of thinner alveolar septa, increased capillary density and functional cell types suggests an evolutionary trajectory optimized for high metabolic efficiency and sustained oxygen uptake. These features emphasize phylogenetic continuity within Caprinae while also highlighting the red serow's adaptation to its specific niche. Baseline anatomical and ultrastructural data generated in this study will also aid in identifying pathological deviations in future veterinary, ecological and conservation research. Despite a limited sample size (n = 3) and the absence of age or sex stratification, the findings provide a valuable reference for subsequent comparative, physiological and conservation-based studies.

In summary, the lung of the red serow exhibits the fundamental histoarchitecture of a typical ruminant lung but with specialized refinements that likely facilitate respiratory efficiency in its rugged montane habitat. These observations contribute new comparative insights into Caprinae pulmonary morphology and serve as a foundational reference for both anatomical and conservation-oriented investigations.

CONCLUSION

In conclusion, it should be noted that even though the general morphologies of the cells and the organelles within them seemed normal, there were some significant ultra-structural characteristics that were very different from those of other species. For example, the cytoplasm of mucous-producing cells contained heterogenous secretory granules that improved the ability to trap dust, pathogens and other airborne particles. Additionally, the phagocytosis of foreign particles and microorganisms entering the lungs is substantiated by the existence of alveolar macrophages. In addition, this study contributes to the establishment of baseline information regarding the histology and ultrastructure of Mizoram Red Serow's lungs.

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Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Informed consent

All animal procedures for experiments were approved by the Committee of Experimental Animal Care and Handling techniques were approved by the University of Animal Care Committee.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish or preparation of the manuscript.

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