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Effect of altitude on localization of certain histoenzymes of the trachea and lungs of Pashmina, Bakerwali and non-descript goats of UTs of Jammu and Kashmir and Ladakh

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Pashmina, one of the most important goat breeds of Ladakh and Bakerwali, is a well established goat breed best known for its migratory habits. Jammu city with an altitude of 327 m to 412 m from msl, shares the same climatic conditions like the rest of North-Western India. Ladakh is the dry temperate region and the highest plateau in the Union territory of Ladakh with an altitude ranging from 3,000 m to 5,000–5,500 m from msl. Both the regions also have a considerable difference in geo-climatic conditions. The knowledge about the biochemical mechanisms which enable high-altitude animals like Pashmina goats to survive and function properly under hypoxic stress environments can provide important information about the nature of physiological adaptation. The non-descript goats is a habitant of the plain region of low altitude regions of Jammu.

Anatomical study on the respiratory system has been conducted in domestic mammals by various workers (Hare 1975) in horse, Suman *et al.* (2005) in goat, Baba and Choudhary (2008) in Black Bengal goat, Kumar *et al.* (2013) in sheep and Danacu *et al.* (2015) in goat. Goat shows distinctive organization of respiratory organs as compared to large ruminants (Kalita 2014).

Comparative morphological studies on localization of histoenzymes of the trachea and lungs in animals living in regions with varied altitudes and geo-climatic conditions are scant. Hence, the present work was designed to study the possible variations of localization of certain enzymes in the lungs of Pashmina, Bakerwali and non-descript goats of the Union Territories of Ladakh and J&K as these are three important breeds of goats which are the normal habitants of Ladakh and Jammu region.

Fresh unfixed trachea and lungs tissues from all the three breeds of goat were collected and placed in optimal cutting temperature (OCT) compound and frozen in liquid nitrogen. Cryostat sections of 10 µm thicknesses at –20°C were obtained on glass slides and incubated with different substrates for the demonstration of phosphatases, viz. alkaline phospharase (AKPase); oxidoreductases, viz Succinic dehydrogenase (SDH), Lactate dehydrogenase (LDH) and Glucose-6-phosphate dehydrogense (G-6-PD) and Diaphorases, viz. Reduced nicotinamide adenine dinucleotide phosphate (NADPH) as per Pearse (1972).

Alkaline phosphatase: Phosphatases are present in a wide variety of animal tissues. They are responsible for the hydrolysis of organic phosphate esters (Sangha and Guraya 1989). Alkaline phosphatase and acid phosphatase are lysosomal enzymes which catalyze various reactions in the body and are involved in the active transport of protein and DNA turnover in nucleus (Mishra *et al.* 2003). Alkaline phosphatase exhibits optimal activity at high pH values while acid phosphatase exhibits optimal activity at low *p*H values (Bancroft 1975).

In the adult mammal, alkaline phosphatase is present in most tissues, but high activities are found only in a few; thus, the small intestine, kidney, bone, and placenta have specific activities, one to two orders of magnitude above those of other organ (Moss and Handerson 1999). In the present study, the AKPase activity was weak in the tracheal epithelium and moderate to strong in the alveoli of lungs in Pashmina and Bakerwali goats (Fig. 1). However, the activity of this enzyme was weak to moderate in case of alveoli of non-descript goat (Fig. 2). Our findings are supported by the findings of Suthakar *et al.* (2008) who reported that the usual alveolar epithelium did not contain alkaline phosphatase, but the cuboidal alveolar epithelium contained some of this enzyme. Suthakar *et al.* (2008) have also reported that the surface epithelium of primary bronchi showed moderate reaction to acid and alkaline phosphatases in guinea fowl.

Glucose-6-phosphate-dehydrogenase (G-6-PD): G-6-PD has been reported to alter the supply of energy to the cells (Zhang *et al.* 2000). In this study, the epithelial lining of trachea showed strong reaction to glucose-6-phosphate dehydrogenase in Bakerwali (Fig. 3) and non-descript goats, and moderate in Pashmina goat (Fig. 4). The reaction of G-

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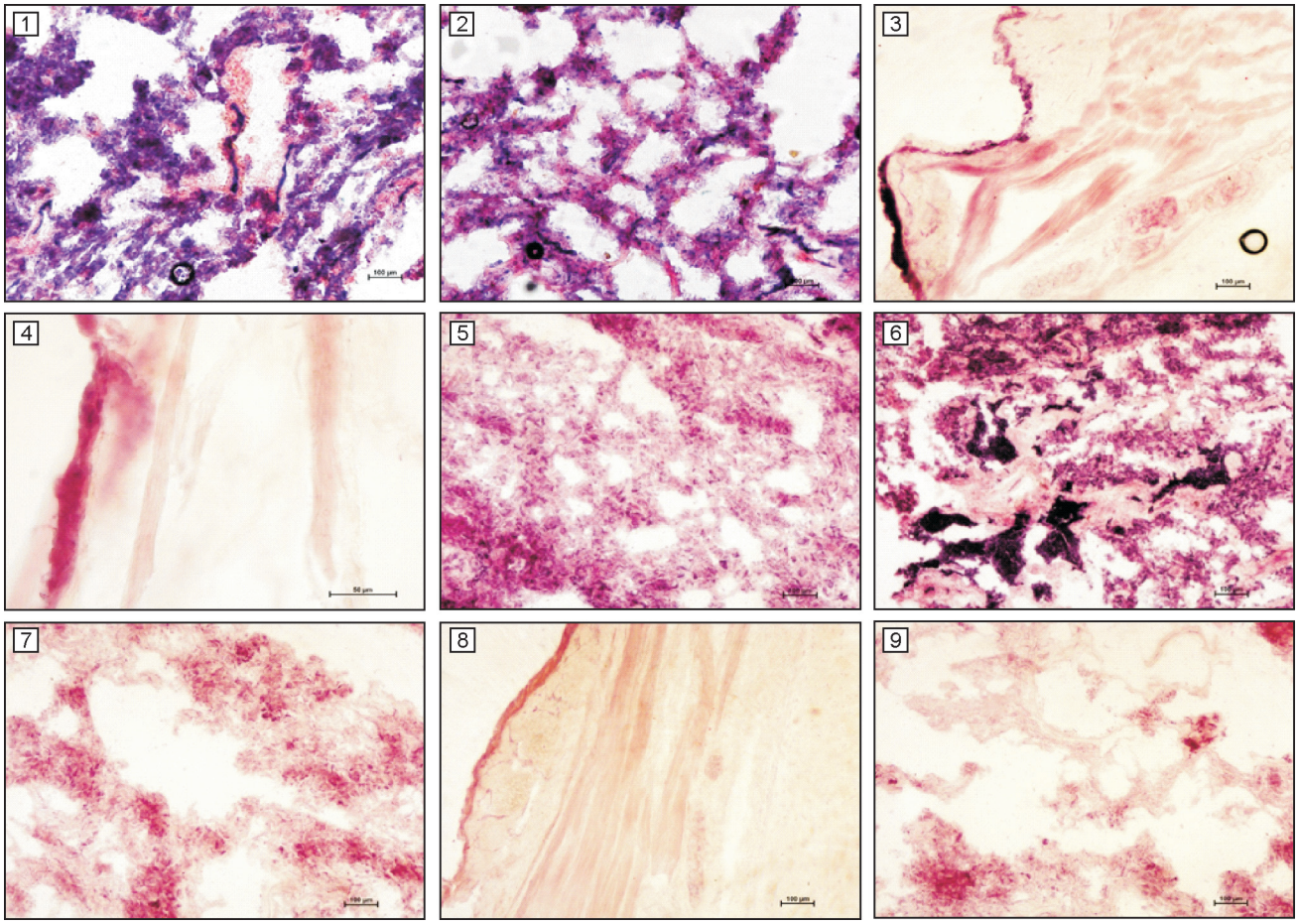
6-PD was recorded as moderate in the alveoli in the Bakerwali goat (Fig. 5), but it was intense in the alveoli as well as bronchi of non-descript goat (Fig. 6). However, a weak reaction was observed in the alveolar epithelium in case of Pashmina goat (Fig. 7). A relatively less activity as seen both in the tracheal and pulmonary tissues in Pashmina goat might be due to the hypoxic high altitude and extremely cold environment as they are their natural habitats. Peter *et al.* (1994) reported that the epithelia of the respiratory and gastrointestinal tract contain a distinct population of disseminated cells called brush cells or caveolated cells that display strong immunoreactivity for nitric oxide synthase (NOS) and also exhibit high activity of NADPH diaphorase. NADPH, in turn, appears to be delivered by glucose-6-phosphate dehydrogenase, which was found in brush cells at particularly high levels, because G6PD is a major NADPH generating enzyme. Also, it was assumed that the high activity (amount) of G6PD in brush cells serves to fuel NOS with NADPH.

Lactic acid dehydrogenase (LDH): LDH is a glycolytic enzyme (it catalyzes the reversible conversion of lactate in the presence of NAD^+ to pyruvate and $NADH^+$) in conditions of glycolysis and is found in almost every tissue, especially in skeletal muscle, heart, liver, kidneys, brain, lungs, and red blood cells (Brian *et al.* 2013). They also act as biomarkers for monitoring of disease activity in cases of hemolytic and megaloblastic, lung diseases and some tumors, particularly lymphomas and germinal cell cancers (Jorge *et al.* 2013). In our present study, the tracheal epithelium showed moderate reaction to LDH in Bakerwali goat (Fig. 8). However, the weak reaction was observed in the cartilage surrounding the bronchi in Pashmina and non-descript goats. Moderate to strong reactions were observed in lining epithelium of bronchi, perichondrium and alveolar epithelium. The alveolar epithelium showed strong reaction to LDH in the Pashmina goat (Fig. 9). The level of LDH



Figs 1–9. **1**. Cryostat sections of lungs showing moderate to strong reaction of alkaline phosphatase in the healthy Bakerwali goat. Azo dye method, 100×. **2**. Cryostat sections of lungs showing weak to moderate reaction of alkaline phosphatase in the healthy non-descript goat. Azo dye method, 100×. **3**. Cryostat section of trachea showing strong reaction of G-6-pase in the healthy Bakerwali goat. Nitro BT method, 100×. **4**. Cryostat section of trachea showing moderate reaction of G-6-pase in the healthy Pashmina goat. Nitro BT method, 100×. **5**. Cryostat sections of lungs showing moderate reaction of G-6-pase in the healthy Bakerwali goat. Nitro BT method, 100×. **6**. Cryostat sections of lungs showing intense reaction of G-6-pase in the healthy non-descript goat. Nitro BT method, 100×. **7**. Cryostat sections of lungs showing weak to moderate reaction of G-6-pase in the healthy Pashmina goat. Nitro BT method, 100×. **8**. Cryostat section of trachea showing moderate reaction of LDH in the tracheal epithelium of healthy Bakerwali goat. Nitro BT method, 100×. **9**. Cryostat sections of lungs showing moderate to strong LDH reaction in the alveoli of lungs in Pashmina goat. Nitro BT method, 100×.

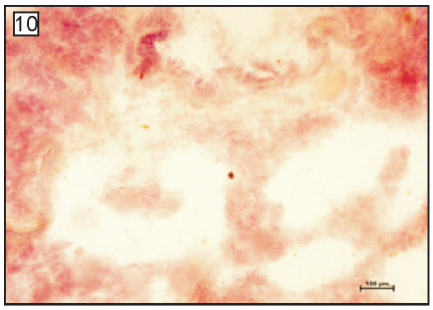
activity and the functional properties of this enzyme indicated the capacity for anaerobic energy production and, hence, the level of resistance to oxygen deficiency during the conditions like hypoxia, vigorous exercise or thermal stress (Portner 2002, Somero 1998). Strong LDH activity as observed in the alveolar epithelium in Pashmina goat is attributed to anaerobic energy production i.e. conversion of lactate in presence of NAD^+ to pyruvate and $NADH^+$ more efficiently as compared to Bakerwali and non-descript goats. Such efficiency might be due to their adaptation to the extremely cold and hypoxic conditions of their natural habitats in Leh and Ladakh regions of Jammu and Kashmir state.

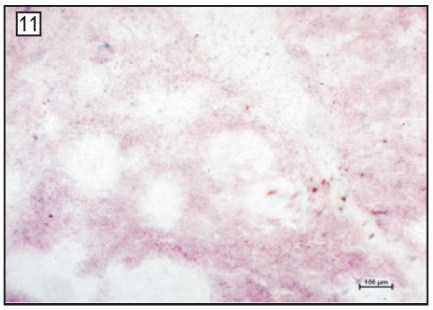
Succinic acid dehydrogenase (SDH): A strong reaction for succinic acid dehydrogenase enzyme was observed in the epithelial lining of trachea in Pashmina goat. However, in Bakerwali and non-descript goats, it showed weak reactions. Moderate reaction of SDH was observed in the alveolar epithelium of lungs in case of Pashmina and Bakerwali goat (Fig. 10), while, there was a weak reaction of SDH in alveolar epithelium of non-descript goat (Fig. 11). Stronger SDH reactions as observed in the tracheal epithelium and pulmonary issues in Pashmina goat as compared to those of Bakerwali and non-descript goats indicated more cellular activities of Pashmina goat. Such enhanced activities might be due to their high altitude habitats. Suthakar *et al.* (2008) also reported that the surface epithelium of primary bronchi showed moderate reaction to succinic acid dehydrogenase in guinae fowl. However, no more literature was found to compare with the present findings.

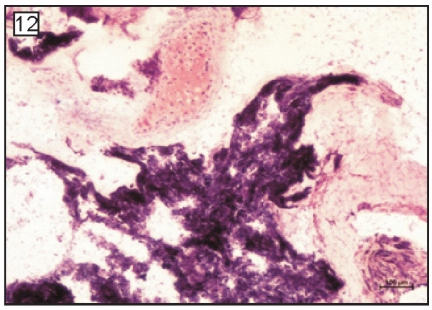
NADPH: In the present study, the tracheal epithelium, bronchial cartilage and the alveoli of lungs showed an intense reaction to NADPH in non-descript goat (Fig. 12) and moderate to strong reactions was observed in Pashmina and Bakerwali goats (Fig. 13). Peter *et al.* (1994) had reported that the epithelia of the respiratory tract contain a number of disseminated cells called brush cells or caveolated cells that exhibit high activity of NADPH-diaphorase. This was in corroboration to our findings in non-descript goats. They further stated that NADPH, in turn, seemed to be delivered by glucose-6-phosphate dehydrogenase, which was found in brush cells at particularly high levels, because G-6-PD is a major NADPH generating enzyme. This phenomenon was again in agreement to our findings in non-descript goats.

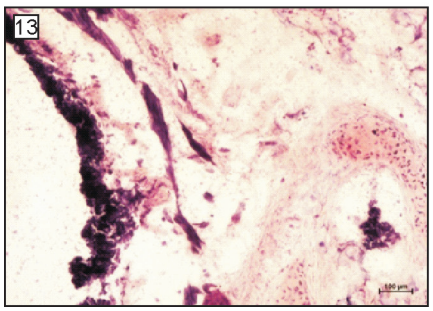
SUMMARY

Comparative studies on some histoenzymic entities were conducted on the trachea and lung tissues of adult Pashmina, Bakerwali and non-descript goats (n=10 each) inhabiting at different altitudes and geo-climatic conditions of Ladakh and Jammu and Kashmir. The study was conducted in Division of Veterinary Anatomy, Faculty of Veterinary Science and Animal Husbandry, R S. Pura, Jammu in 2019. A part of the same was also conducted in the Department of Veterinary Anatomy and Histology, GADVASU, Ludhiana. Tissues from these organs were subjected for localizing certain tissue enzymes, viz. Alkaline phosphatase (AKPase), Glucose-6-phosphate-dehydrogenase (G-6-PDH), Lactic acid dehydrogenase (LDH), Succinic acid dehydrogenase (SDH) and NADPH. The epithelial lining of trachea showed strong reaction to G-6-Pase and LDH in Bakerwali goat, while SDH showed strong reaction in Pashmina goats. Again, in regard to lung tissues, AKPase and LDH showed moderate to strong reactions in Pashmina goat, but SDH exhibited strong reactions. In Bakerwali goats, AKPase and LDH showed moderate to strong reactions, while in non-descript goats, LDH exhibited moderate to strong reactions and G6PDH showed intense reactions. This revealed that not much variations were observed in regard to localization of certain histoenzymic entities in trachea and lung tissues of three breeds of goats which are the inhabitants of different altitudes and geo climatic conditions.









Figs 10–13. **10**. Cryostat section of lungs showing moderate reaction of SDH in the alveolar epithelium of lungs in healthy Bakerwali goat. Nitro BT method, 100×. **11**. Cryostat sections of lungs showing weak reaction of SDH in alveolar epithelium of healthy non-descript goat. Nitro BT method, 100×. **12**. Cryostat section of lungs showing intense reaction of NADPH diaphorase in alveoli and bronchial cartilage of non-descript goat. Nitro BT method, 100×. **13**. Cryostat section of lungs showing strong reaction of NADPH diaphorase in alveoli and bronchial cartilage of Pashmina goat. Nitro BT method, 100×.

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