Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2018; 6(3): 1141-1148
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Received: 06-03-2018
Accepted: 07-04-2018

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Light and scanning electron microscopy aided with elementary analysis in characterization and identification of hair of Assam hill goat

Sukanta Das, Kabita Sarma, Manmath Talukdar, Jiten Rajkhowa, Chandan Gautam, Snehanshu Sinha and Anil Deka

Abstract
The present investigation was carried out in the Assam Hill Goat (*Capra hircus*) to investigate some morphological and numerical features of hair of the Assam Hill Goat by light and scanning electron microscopy aided with elementary analysis for identification. The animals were divided in three groups viz. preruminant (0-3wks), transitional (3-8wks) and ruminant (above 8 wks). The morphometrical study of hair was conducted by direct examination and scale cast method. Light microscopy of hair below the skin of Assam Hill Goat in H &E stain showed the presence of continuous, vacuolated, imbricate lattice pattern of medulla with wavy margin, cuticle as transverse scale with an oblique angle to the long axis of hair and homogenous cortex. The diameter of hair shaft, cortex, and medulla were found to be significantly higher in ruminant age groups goat. Various indexes (Cortical, medullary, cuticular indexes) can be used as the identification criteria of the Assam Hill Goat. The scale count/100 micrometer was found to be significantly high in ruminant age groups. In direct examination and scale cast method medulla was found as uniseriate/multisireate, continuous and partially filled lattice, with scalloped margin, cuticle was transverse, with smooth rippled margin and regular to irregularly flattened and with homogenous with little brown pigment in all the age groups. The Scanning Electron Microscopy revealed primary hair shaft was encircled by 2-4 numbers of scales with the rough appearance wider diameter than wool hair. Medulla was composed of cavities and irregular space with uneven walls. The scale distance was found to be reduced with the advancement of age. The elementary analysis of the hair of the Assam Hill Goat for the various elemental spectra (C, O, S, Na, Ca, K, Co, Cu, Fe, Mn, Mg, Zn) can be an indicator of geographical location for identification of the Assam Hill Goat. EDS study showed that the carbon, oxygen and sulpher were the main elements of hair of the Assam Hill Goat.

Keywords: Hair, SEM-EDS, SEM, Assam hill goat, scale cast

Introduction
Hair plays an important role in various fields counting taxonomy, zoology, evolution, wildlife clinical biology, dermatology, race, sex age, occupational identification, archaeological study and forensic investigation (Sahajpal [1], Meyer and Schnapper [2]; Farag and Abou [3] and National Research Council [4]. Hair being to its non-invasive, easy collection and quick analysis along with précised and accurate results it can be preferred as an important biological sample. Hair examination was in routine practice since last decades for species identification as well as for clinical detection of various diseases Goyal and Sahajpal [5]. Various morph metric parameters, of hair architectures can provide many information (Chernova [6], Monica and Peric [7]). The Scanning electron microscopy coupled Energy Dispersive Spectra (EDS) which will leads to the identification of geographical region by elemental analysis can be an added advantage. As there is paucity of literature regarding the identification study of hair of the Assam Hill Goat. So a comprehensive attempt is made in order to establish the norms for the identification of hair of the Assam Hill Goat.

Materials and Methods
In the present investigation the hair and the skin sample (4-5mm) were collected from the eighteen numbers of Assam Hill Goat during the preruminant (0-3wks), Transitional (3-8wks) and Ruminant (8wks above) age group, randomly irrespective of sex and coat color. Skin samples were fixed in 10% neutral buffered formalin followed by routine paraffin section and H&E staining as per Luna [8].

Morphological Investigation of hair was done by the whole mount method and scale cast method as per the Chattha and Anjum [9], then viewed at various Magnification. Micrometrical readings were taken like shaft diameter, diameter of the medulla and cortex. Various micrometrical studies were done by means of standard methods of micrometry using Nikon E 200 camera mounted microscope and Image Pro Express Ver-2.0 Software and analyzed by standard statistical methods. For Scanning Electron Microscopy skin samples were fixed in karnovskys fluid and then processed as per the standard procedure described by NEHU, SAIF shillong, Meghalaya and viewed at various magnifications. X-ray Micro analysis (EDX) for carbon, oxygen, sodium, sulpher, calcium, cobalt, and copper both weight and atomic percentage at Institute of Advanced Study in Science and Technology, Vigyan Path, Paschim Boragaon, Garchuk, Guwahati, Assam

(The Research work was carried out as per the approval of the Institutional Animal Ethics Committee, Approval No: 770/ac/CPCSEA/FVSc/AAU/IAEC/16-17/373, Assam Agricultural University: Khanapara, Guwahati-781022)

Results and Discussion

Histomorphological investigation of the hair in all the three age group showed that the basic hair shaft was composed of cuticle, cortex and medulla from outside to inside (Fig: 1, a, b, c). which is in support with Meyer and Schnapper [2], Anna Maria De Marinis [10] in sheep and goat, Lungu [11] in cattle goat and sheep, Aris and George [12] in goat, Jitendra Gharu [13] in sheep, goat, cattle, Monica [7] in Dairy goat. The medulla was characterized by a central core of continuous, vacuolated, sinuous cellular compartment with imbricate pattern and wavy margin. Lattice pattern was disposed transversely with respect to the longitudinal axis of the hair. Medulla occupied nearly entire width of hair shaft in all the age groups (Fig. 1, 2). The cuticle was characterized by transversely directed scales and arranged at an oblique angle with respect to the longitudinal axis of the hair (Fig: 2 a, b, c). Similar observations were also recorded by Lungu [11] in goat, Verma [14] in baby goat. Chernova [15] reported that variation in the medulla structure can be correlated with adaptation (thermal insulation) in animals. In the present study in all the age groups the hair medulla was characterized by unicellular/multicellular, uniseriate/multiseriate, continuous partially filled lattice, with scalloped margin. Multicellular patterns were common in higher age groups. Jitendra Gharu [13] reported that medulla in goat (*C. hircus)* was simple and continuous, broad with irregular (wavy or crenate) margins. Verma [14] reported unbroken lattice occupying the entire width of the hair shaft in baby goat. The hair scales were arranged in a herringbone pattern, transversal, smooth, distant and regularly flattened. The cuticle structure of scale margin did not show any much variation in preruminant and transitional age group but in ruminant age group scale margin showed smooth and rippled pattern. Similar observations were also recorded by Anna Maria De Marinis *et al.* (2006) [10] in sheep and goat. Mukherjee P. *et al.* (2016) [16] and Jitendra Gharu *et al*. 2015[13] reported that the cuticular margins of hair as imbricated, mosaic with irregular wavy in domestic goat (*Capra hircus*). Anwar [17] explained the cuticular pattern in goat as crenate, continuous in sheep. Cortex was light colored visible with regular distributed pigments. (Fig. 2 a, b, c, 3, 4, 5). Similar observations were also recorded by Lungu [11] in goat and Verma [14] in baby goat, Anwar [17] in sheep. But the cuticular patterns were not clearly distinct in the transitional and ruminant age groups in the present study (Fig. 2 a, b, c) (Fig. 3, 4, 5).

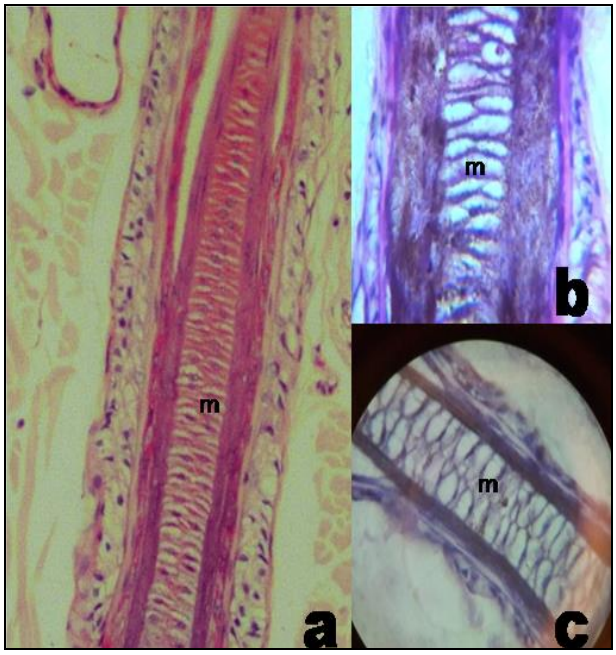


Fig 1: Photograph showing the basic structure of hair shaft at the follicular level during preruminant (c) transitional (b) and ruminant (a) age groups at 400X magnification (H&E)

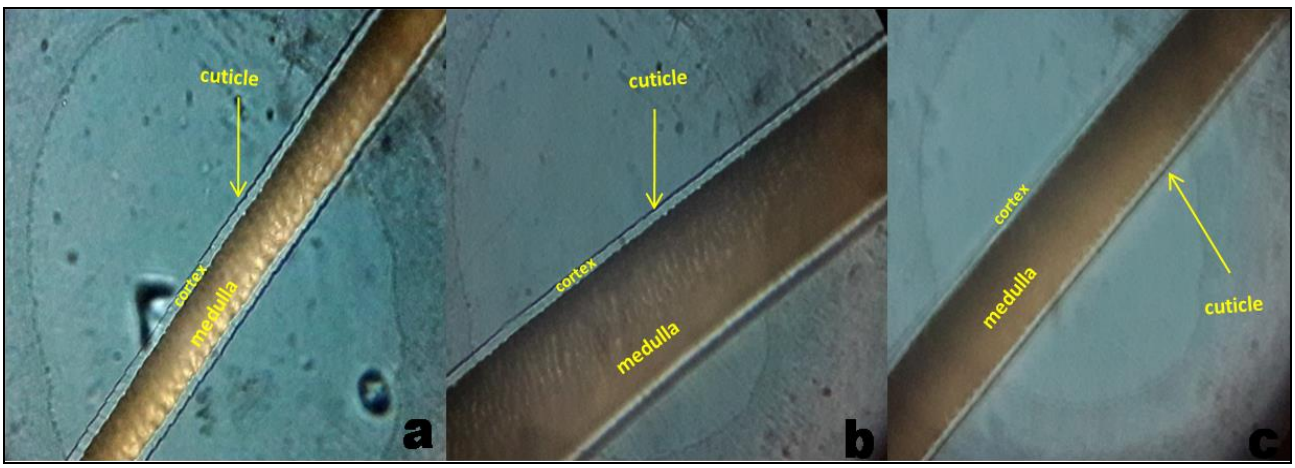


Fig 2: Photograph showing the basic structure of hair shaft of Assam Hill Goat showing the central core of medulla, thin cortex and cuticular margin at the free part during preruminant (a) transitional (b) and ruminant (c) age groups at 400X magnification (Direct Examination)

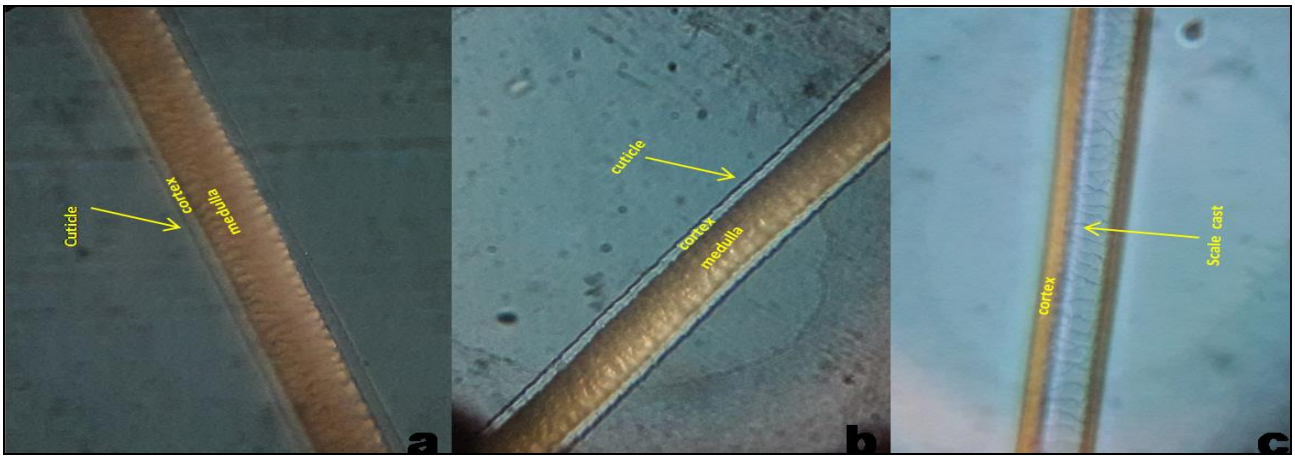


Fig 3: Photograph showing the basic structure of hair shaft showing Assam Hill Goat the during preruminant age groups by direct examination (a, b) and scale cast (c) at 400X magnification

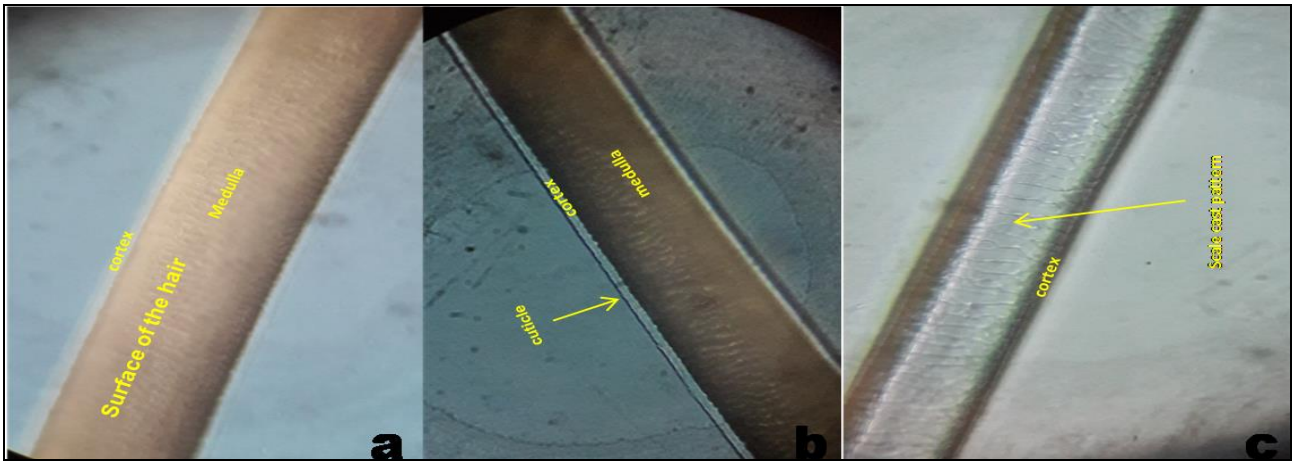


Fig 4: Photograph showing the basic structure of hair shaft showing Assam Hill Goat transitional age groups by direct examination(a,b) and scale cast (c) at 400X magnification

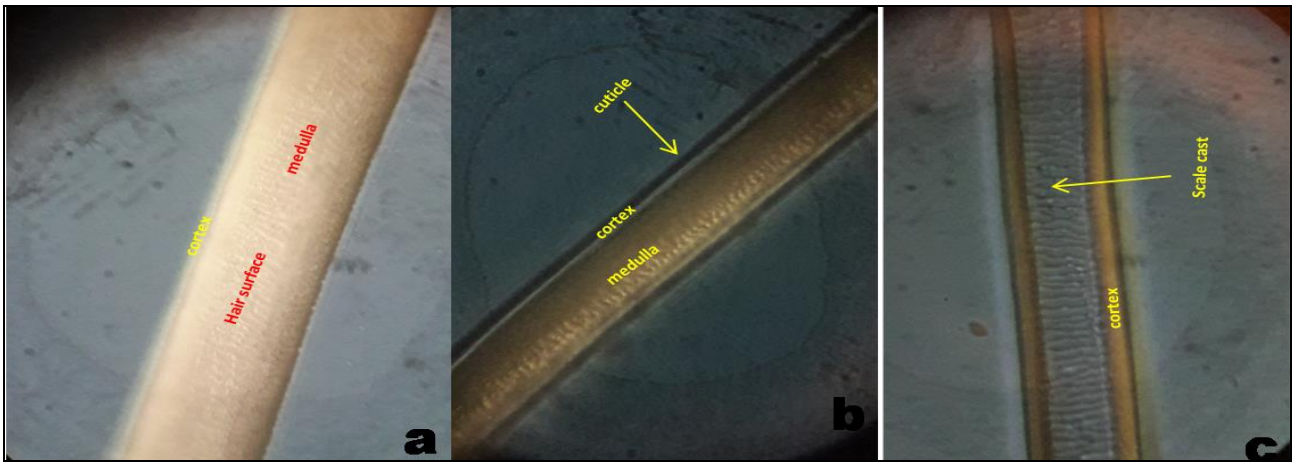


Fig 5: Photograph showing the basic structure of hair shaft showing Assam Hill during Ruminant age groups by direct examination(a,b) and scale cast (c) at 400X magnification

Various micrometrical studies and measurements of hair of Assam Hill Goat (*Capra Hircus*)

Micrometrical studies of hair of Assam Hill Goat revealed that shaft diameter, breadth of cortex and medulla of hair shaft were significantly higher in ruminant age group than preruminant and transitional (Table.1). But Aris and George (2008) [12] reported the diameter of wool hair 10-12 micrometer and gaurd hair 45-130 micrometer. Lungu, A. *et al.* (2007) [11] reported the diameter of hair 128.0 (± 16.6) µm in goat. Verma [14] reported the diameter of hair 21-210 micron in baby goat.. The variation in the diameter during the various age group in the present study may be due to nutritional and metabolic factor. Kshirsagar [18] reported variation in the medulla diameter amongst the various species and reported the medulla diameter of Goat 50 µm (SD-5.40), comparable to sheep and buffalo. He also reported that medulla diameter is an important parameter to differentiate animal and human hair. Siddique [19] reported that the hair diameter of Black goats was increased with the age. Considering the above literature it can be reported that the diameter of medulla varied with the age.

The mean cuticular diameter were significantly higher in transitional and ruminant age groups (Table. 1). Scale distance was significantly higher in preruminant. Medullary, cortical and cuticular index did not show much significant difference amongst the age group (Non-Significant, Table. 1) the index values of hair can be used as an important identification feature of Assam Hill Goat. Similar interpretation were also drawn by Gaudette [20], Deedrick and Koch [21], Cadar [22] in dog, Anwar [17] in camel yak sheep goat, Farag [3] wild animals. Though Lungu [11] reported the medullary index in goat was of 0.71. Scale count/100 micron of hair shaft showed significantly lower in preruminant age group, whereas in transitional and ruminant age group was not significant Aris and George [12] reported that scale count can be important criteria for identification of species. These photographic evidences about the morphology of hair of Assam Hill Goat particularly various indexes, pigmentation pattern, shape of cuticular scales and features of medullar cells can be used as reference. similar observations were also recorded by Meyer [2], Chernova [6] in mammals; Monica [7] in dairy goat, Aris and George [12], Anna Maria De Marinis [10], Lungu [11] wild ruminant Kshirsagar [18], Anwar [17] in goat, yak, sheep, zomo, Cadar [22] in various breeds of dogs, Farag [3] in wild animals.

Table 1: Micrometry of various parameter of hair of Assam Hill Goat

	Preruminant	Transitional	Ruminant
Parameters of hair follicle	**Mean ± SE**	**Mean ± SE**	**Mean ± SE**
Diameter of hair shaft (***)	72.083^{a}±2.776 µ	94.33^{a}±3.887µ	124.166^{b}±9.782 µ
Breadth hair cortex (**)	7.083^{a}±0.351 µ	8.083^{a}±0.238 µ	10.083^{b}±0.675 µ
Diameter of hair medulla(***)	62.500^{a}±2.77 µ	82.33^{a}±3.729 µ	110.166^{b}±9.30 µ
Diameter of hair cuticle (**)	2.50^{a}±0.63 µ	3.916^{b}±0.37 µ	3.58^{b}±0.73 µ
Scale distance (***)	9.00^{a}±1.546 µ	6.65^{b}±0.864 µ	5.135^{b}±1.581 µ
Medullary Index (X100) (ns)	86.62*±2.15 µ	87.195*±1.123 µ	88.54*±1.973 µ
Cortical index (X100) (ns)	9.885*±0.57 µ	8.626*±0.406 µ	8.226*±0.511 µ
Cuticular index (X100) (ns)	3.476*±0.362 µ	4.168*±0.159 µ	3.926*±0.0315 µ
Scale count/100 µ (***)	25.66^{a}±1.22	50.50^{b}±2.78	54.16^{b}±2.38

Means within the same row in each item within each group carrying different superscripts are significantly different at (p<0.05),

Scanning Electron Microscopy of Hair

The scanning electron microscopy of hair of Assam Hill Goat showed the scale like overlapping cuticle (Fig, 8 a, b, c). Core of the hair was formed by the medulla (Fig 6, 7). Diameter of the wool hairs were less than the guard/primary hairs. (Fig. 6, 7) These findings were in accordance with Pourlis [12] in goat (*Capra prisca*). The wool hairs have a uniform appearance (Fig. 9). The cuticular pattern was non-annular type with scales adhered closely to the shaft oriented towards the apex of the hair, non-annular overlapping flattened with smooth margin, wide breadth and less height (Fig, 8, 9, 10). Similar findings were also reported by Pourlis [12] in goat and he reported rippled margins of the scale in *Capra prisca* also reported by Jitendra Gharu [13] in goat. Pourlis [12] reported an intermediate scale pattern in the guard hair and primary hair with mixed scale pattern and mixed wave shape and reported may be an distinguish characteristics of the breed in *Capra prisca*. In the present study the primary hair shaft was encircled by 2-4 numbers of scales with wavy and smooth margin (8 a, b, c) findings were in support to Pourlis [12] in goat. De Marinis and Asprea [10] reported that the cuticular pattern varies among breed. Inagaki [23] and Bakuneeta [24] used scale patterns to identify chimpanzee hair by Scanning electron microscopy. Hairs had a round profile in cross section with porous medulla. Medulla was composed of cavities and irregular space with uneven walls (Fig. 7, 6). Pourlis [12] in goat reported the medulla was composed of cavities with uneven walls with prickly process. The features of medulla can be used as reference for identification of the Assam Hill Goat which is in agreement with Clement [25] and Inagaki [23]. The mean scale distance of wool hair and primary hair during preruminant transitional and ruminant age group 9.0375±0.56143µ, 6.7±0.301 µ and 6.589±0.301 and 6.9575±0.44µ, 3.76±0.244 µ and 3.7075±0.3636 µ respectively Dahiya [26] stated that the scale distance in Asiatic Lion, tiger leopard can differentiate each other within the family

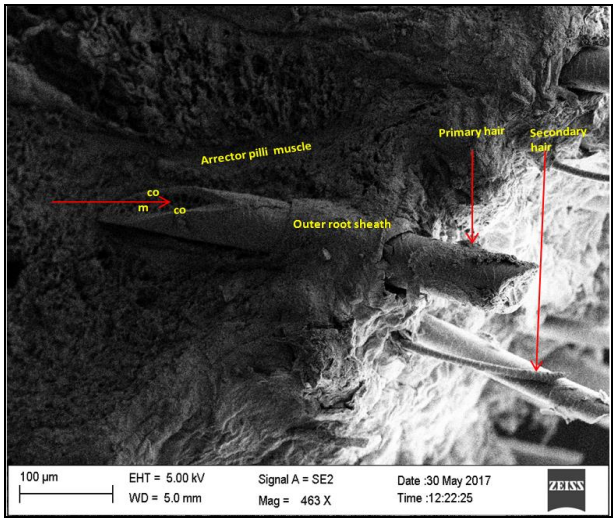


Fig 6: Photograph showing the electron microscopic feature of the hair of Assam Hill Goat with porous medulla an thin cortex

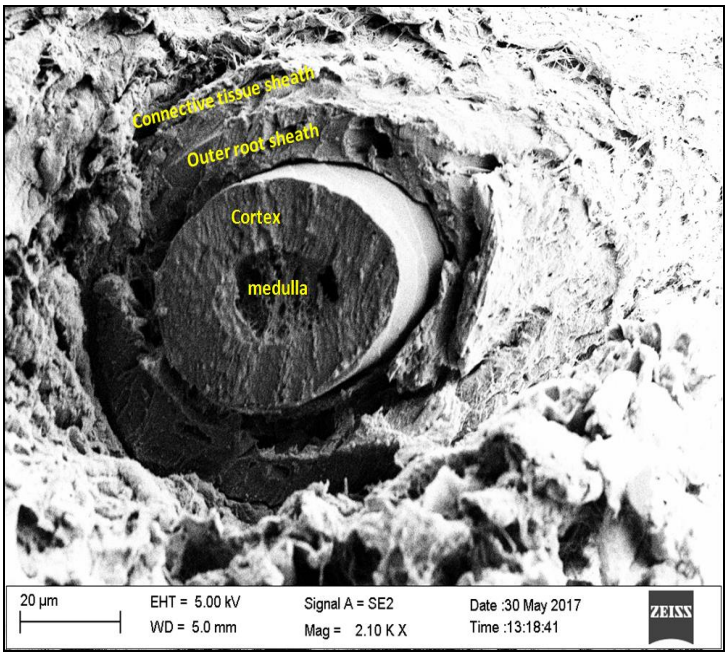


Fig 7: Photograph showing the cross sectional profile of hair of Assam Hill Goat at Scanning electron microscopy

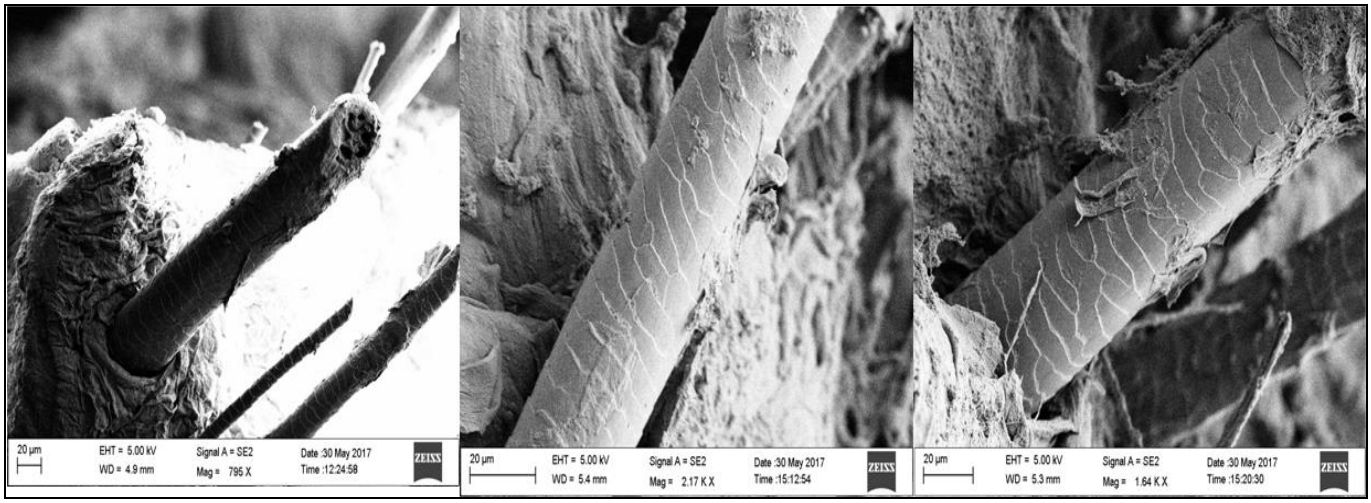


Fig 8: Photograph showing the hair surface with transversely directed scales of Assam Hill Goat during preruminant (a), Transitional and ruminant (c) age groups

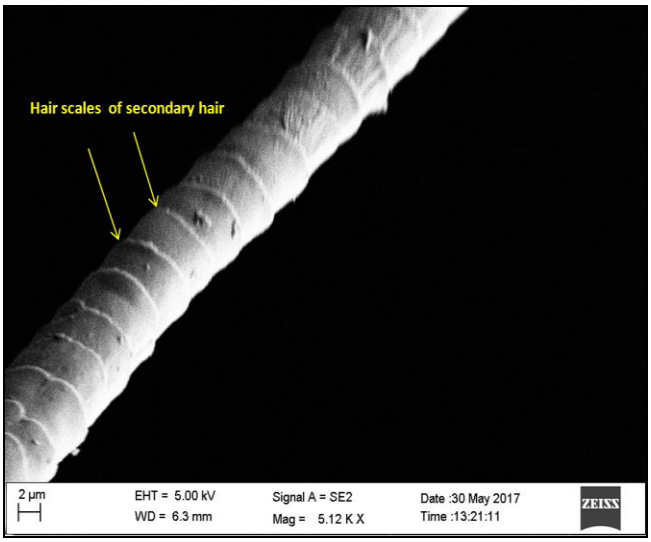


Fig 9: Photograph showing the hair surface wool hair with smooth profile of Assam Hill Goat

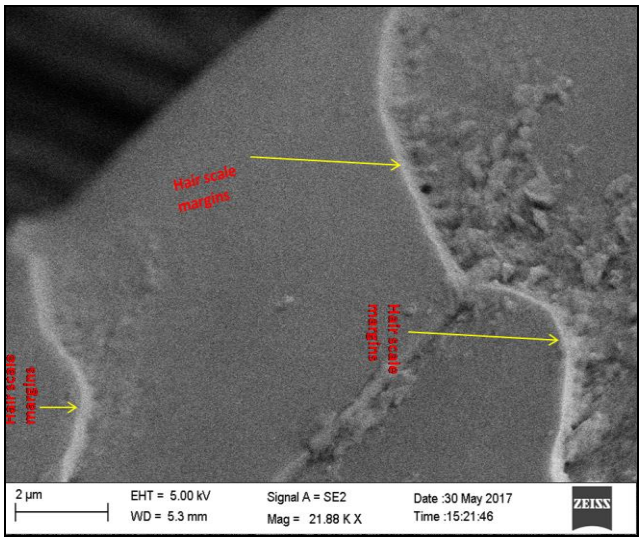
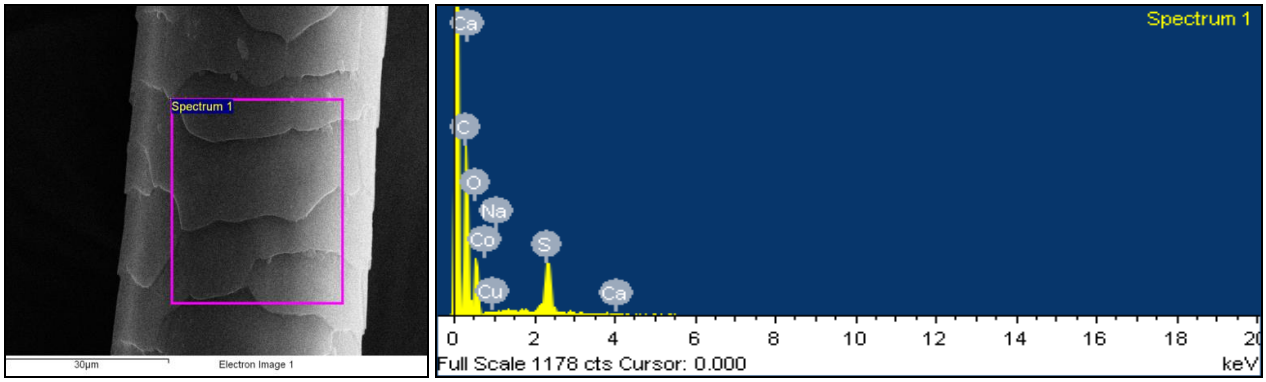


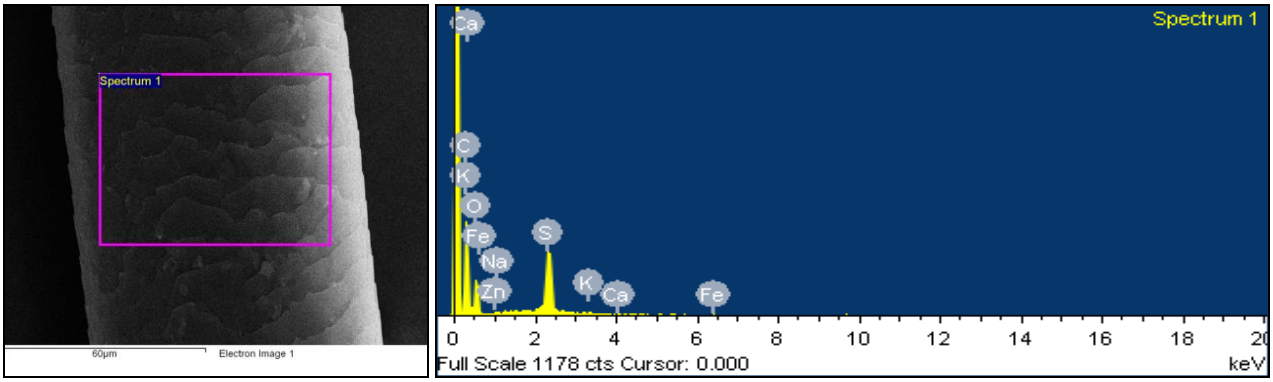
Fig 10: Photograph showing the scales of primary hair Assam Hill Goat

SEM-EDX analysis of Hair: The SEM-EDX (Scanning electron microscopy-elementary dispersive x-ray spectroscopy) elemental analysis of hair of Assam Hill Goat During preruminant, transitional and ruminant age groups reveals that the carbon and oxygen and sulpher were found to be more in quantity in all the age groups of Goat whereas Na and Mg were less. The carbon content with the advancement of age get increased which may be due to loss of inorganic material from the hair, followed by a subsequent increase of organic materials. Oxygen is the second highest quantity present in goat hair, when compared with the other elements. (Table.2) Similar pattern was also reported by Mujeeb [27] in human hair. Significantly higher sulpher was found in ruminant age groups. In the present investigation the average weight % of Na,Ca, K, Co, Cu, Fe during preruminant transitional and ruminant age groups can be compared (Non-Significant). General elemental pattern associated with structure of carbon, oxygen, sulphur, sodium, copper, calcium, potassium, cobalt, and iron can be important for the identification of of the Assam Hill Goat and the geographical location. Dahiya [26] reported that Elemental Analysis of Hair as a tool in identification of Felidae animals in lion leopard and tiger. Choudhary [28] reported that the scanning electron microscopy with the elementary analysis of the hair can be an important criteria for the identification of wild and other animals.

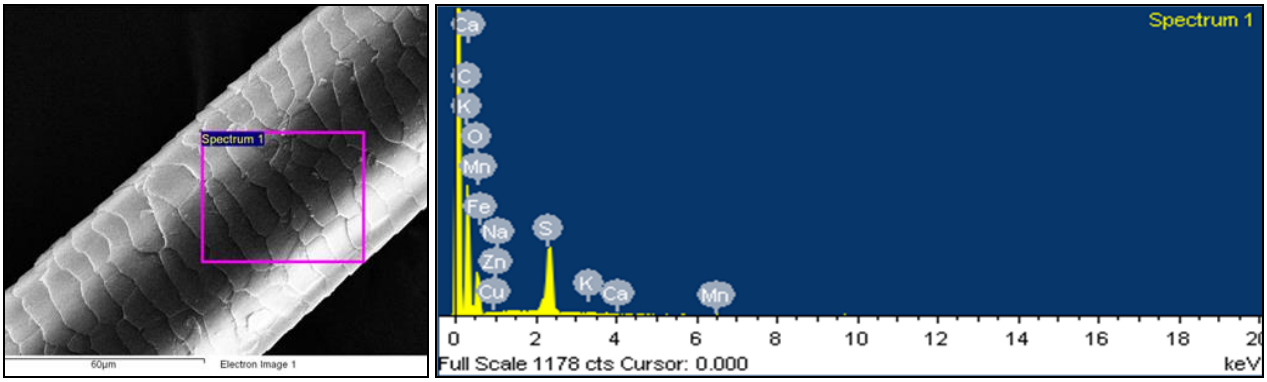
Phographs Showing the Various Mineral Spectra of Hair of Assam Hill Goat by Eds



Elements spectra of hair during Preruminant age group of Assam Hill Goat (SEM-EDS)



Elements spectra of hair during Transitional age group of Assam Hill Goat (SEM-EDS)



Elements spectra of hair during Ruminant age group of Assam Hill Goat (SEM-EDS)

Summary and conclusion

The histomorphological and direct microscopic examination of in the present investigation revealed the general architectures of the hair of Assam Hill Goat (medulla, cortex and scale patern). In addition to that the various indexes, like cortical, medullar and cuticular can be used as the reference to identify the hair of Assam Hill Goat. Though within the same caprine family some similarities were observed but with the aid of SEM-EDS (elementary spectrum) indicating the geographical location obtained by Scanning electron microscopy can provide specific information about the animals.

Table 2: Mean ±Se of different trace mineral of hair during preruminant, transitional and ruminant age groups of Assam Hill Goat by SEM-EDX

	Preruminant	Transitional	Ruminant
Parameters	**Mean ± SE (weight %)**	**Mean ± SE (weight %)**	**Mean ± SE (weight %)**
C (ns)	64.21±0.83*	65.48±0.415*	65.38±0.7238*
O (*)	30.51±0.991[a]	26.91±0.447[b]	26.95±0.3493[b]
S (***)	4.12±0.049 [a]	6.646±0.103 [b]	6.616±0.2587[b]
Na (ns)	0.08±0.040*	0.116±0.020*	0.103±0.0088*
Ca (ns)	0.063±0.003*	0.083±0.003*	0.07±0.0208*
K (ns)	0.12±0.005*	0.153±0.014*	0.11±0.0152*
Co (ns)	0.11±0.015*	0.14±0.02*	0.15±0.0346*
Cu (ns)	0.16±0.071*	0.22±0.081*	0.143±0.0983*
Fe (ns)	0.22±0.035*	0.12±0.062*	0.126±0.0348*
Mn (ns)	0.046±0.008*	0.033±0.0033*	0.096±0.029*
Mg (ns)	0.046±0.003*	0.043±0.006*	0.063±0.006*
Zn (ns)	0.083±0.006*	0.026± 0.006*	0.186±0.0959*

Means within the same row in each item within each group carrying different superscripts are significantly different at (p<0.05).

Acknowledgement
The Authors duly acknowledged the Head of the Department of Anatomy & Histology, College of Veterinary Science, Assam Agricultural University, Khanapar, Guwhati Assam, India, Institute of Advanced Study in Science and Technology, Vigyan Path, Paschim Boragaon, Garchuk, Guwahati, Assam, and NEHU Shillong, Meghalaya for proving the facilities of SEM-EDS and SEM

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