Journal of Pharmacognosy and Phytochemistry
Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2019; SP5: 166-169

Ratnesh Kumar Choudhary
Krishi Vigyan Kendra, Kishanganj, Bihar, India

P Das
Department of Veterinary Anatomy and Histology, Faculty of Veterinary and Animal Sciences, West Bengal University of Animal and Fishery Sciences, 37, K. B. Sarani, Kolkata, West Bengal, India

Correspondence
Ratnesh Kumar Choudhary
Krishi Vigyan Kendra, Kishanganj, Bihar, India

(Special Issue- 5)
International Conference on
"Food Security through Agriculture & Allied Sciences"
(May 27-29, 2019)

Studies on skin-associated lymphoid tissue (SALT) of black Bengal goat

Ratnesh Kumar Choudhary and P Das

Abstract
Black Bengal Goat is a meat-type breed, known for high prolificacy, superior meat quality and best quality skin. Due to high mortality and morbidity rates in kids low cost "goatery" is not popularizing. General health and disease susceptibility is directly related with the immune status of the individual. Innate immunity includes the external barriers of the body, like the skin and mucous membranes, which are our first line of defense in preventing diseases from entering the body. Hence the present studies has been undertaken to elucidate with the age wise gross structural development of skin, to identify Langerhans cells of the skin and identify age wise changes of N.K. Cells. Skins were collected from clinically healthy zero day and month wise from one month to fifth months old Black Bengal goats. Skin as a whole the largest organ of the body gives the natural immunity from the date of birth. Stratum lucidum was absent in all age groups. The skin thickness increased gradually with the increment of age. The depth of the sweat gland, hair follicle was increased with age. CD57 antibody against Caprine NK cells antigen was used to identify NK cells, S100 and MHC-II-both immune markers were used to identify APC cells of the skin. The presence of different APC and NK cells and there increment with age revealed the active role of skin to combat the body defense mechanism from date of birth. But initially up to 2nd months there was some less population but from 3rd months the number of APC and NK cells increased rapidly.

Keywords: Black Bengal goat, skin, lymphoid tissue

Introduction
Eastern and north eastern part of India is proud of prevailing very good quality goat breeds like Black Bengal goat. The Black Bengal is a well-known dwarf meat-type breed of goat known for high prolificacy, superior meat quality and best quality skin. General health and disease susceptibility is directly related with the immune status of the individual. Innate immunity includes the external barriers of the body, like the skin and mucous membranes (like those that line the nose, throat, and gastrointestinal tract), which are our first line of defense in preventing diseases from entering the body. If this outer defensive wall is broken (like if you get a cut), the skin attempts to heal the break quickly and special immune cells on the skin attack invading germs.
The skin has the same embryologic origin as the thymus and it too may be a site of T cell maturation (Tizard, 2000) [6]. These cells interact with keratinocytes and can mature within the epidermis. Langerhans cells are dendritic cells found in the epidermis of the skin. However, the skin also functions as an effective antigen- trapping barrier, and these T cells mar also serve a local defense function.
Low cost "goatery" is not popularizing because of the high mortality and morbidity rates in kids. The roles of age-associated changes in the immune system are responsible for an increased likelihood of infection and autoimmune diseases. Hence the present studies has been undertaken to elucidate with the age wise gross structural development of skin, to identify Langerhans cells of the skin and identify age wise changes of N.K. Cells.

Materials and Methods
Skin were collected from clinically healthy zero day and month wise from one month to fifth months old Black Bengal goats (*Capra hircus*) from the farm maintained by AICRP (All India

(All India Coordinate Research project) on goat. The animals were maintained in the farm house according to the stipulated guideline and permission of Institutional Animal Ethical committee of faculty of Veterinary and Animal sciences; West Bengal University of Animal and fishery Sciences, Kolkata. Two goats of either sex were utilized for this experiment in each age group. The Skin samples were taken from neck region in all groups of experimental animals and were fixed in 10 per cent neutral buffered formalin. The fixed samples were processed for paraffin sections and routine staining was done as per Luna, 1968 [3].

For immunohistochemical examination the collected samples were fixed in 10 % neutral buffer formalin (NBF) and were dehydrated through graded alcohols before being embedded in paraffin wax. The sections (horizontal & Vertical) of 5µm thickness were obtained from each specimen.

The different lymphocyte subpopulations were visualized using the avidin-biotin peroxidase complex technique (ABC; Vector Laboratories, U.S.A). Paraffin-embedded samples were dewaxed with xylene and hydrated. Then dip into antigen unmasking (Vector Laboratories, U.S.A) solution for 15 min at 95°C. After washing the slides in PBS for 5 min, the samples were pre-treated with normal rabbit serum (Dako, Denmark) diluted 1:100 in PBS for 60 min at room temperature to block the non-specific binding sites. They were then incubated with the ready-to-use of each primary antibody (Table 1) in a moist chamber for 120 min at room temperature. Next, the slides were again washed in PBS and then incubated with a biotinylated rabbit antimouse IgG or horse anti-rabbit IgG (Vector Laboratories, U.S.A) according to primary antibody diluted 1:250 for 60 min. Before being washed in PBS and incubated again with ABC for 40 min, according to the instructions of the maker (Vectastain ABC Elit® Kit, Vectror Laboratories). Color was developed by a final incubation with 3.3' diaminobenzidine tetrahydrochloride (DAB, Peroxidase substrate kit Vector Laboratories) in buffer with hydrogen peroxide in distilled water for 5 min at room temperature. The reaction was stopped by rinsing the slides in water. The some slides were counterstained with haematoxylin, dehydrated and mounted. Most slides were mounted without counterstained.

Tissue samples for electron microscopy were fixed in 2.5% glutaraldehyde in PBS (0.1M; pH7.0). The standard protocol was followed for TEM (Transmission electron microscopy) and SEM (Scanning electron microscopy). The present study was carried out partly in the department of Anatomy, Histology and Embryology, West Bengal University of Animal and Fishery Sciences and partly at Electron-Microscopic facility, All India Institute of Medical Science, Ansari Nagar New Delhi.

Table 1: Primary Antibodies (ready- to- use) used in the Immunohistochemical Technique

Antibodies	Specificity	Isotype	Supplier
TB01	CD57*	IgM	Dako Denmark
	S100**		Dako Denmark
CR3/43	HLA-DP, DQ, DR*	IgG1	Dako Denmark

*****Monoclonal Antibody, ******Polyclonal Antibody

Result

Histological study

Skin samples were taken from neck region in all groups of experimental animals. The histological sections of skin at birth revealed normal histoarchitectur of epidermis and dermis. The epidermal thickness was recorded as the thinnest among the experimental groups. Stratum corneum was found absent at birth. There was a clear single layer of stratum basale. The nucleus was round to oval in shape and was highly basophilic. Subsequent two layers followed by stratum basale revealed stratum spinosum and shape of nucleus were elliptical. The nucleus of stratum granulosum was lightly basophilic and cytoplasm was eosinophilic. The superficial layer of epidermis revealed small spindle shaped nucleus and the size of nucleus was smaller than the previous layers, which may be considered as stratum superficiale particularly in this age. At the dermo-epidermal junction, the epidermis made a farm attachment with the dermal component by making papillae within the papillary layer of dermal component. There were numerous dispositions of connective tissue fibres in the dermis. The total dermal component was divided into superficial papillary layer and deep reticular layer. But it was very thin in day old goat and there was no clear demarcation between two layers. The reticular layer was thicker and consisted of dense irregular connective tissue fiber and connective tissue cells were scatteredly distributed within these layers. Numerous primary, secondary hair follicles were found right from the date of birth. Small sebaceous glands were located around the hair follicle. The less developed small size sweat glands were found in the upper papillary layer of dermis at birth. From first month onwards the epidermis was clearly comprised of stratum basale, stratum spinosum, and stratum corneum. Stratum lucidum was absent (Fig. 1). The epidermal thickness increased with the increment of age, at 5th month of age it was recorded maximum. Epidermal keratinocytes increased in numbers from 1st month onwards. The shape and size of the sweat and sebaceous glands increased according to advancement of age and the number of hair follicle increased in the same pattern.

Immunohistochemistry

Skin samples from the neck region were considered for identification of natural killer (NK) cells and antigen presenting cells (APC) in different groups of experimental animals. CD57 antibody against Caprine NK cells antigen was used to identify NK cells. CD57 positive cells were identified from birth onward (Fig. 2). Morphologically the cells were round to elliptical in outline and distributed mostly within the connective tissue fibres of the dermis and at the dermoepidermal junction. Regarding the frequency of distribution with age it was observed that there was no noticeable change in terms of frequency throughout the experimental period. However in some particular slides the cells were found to be distributed in the papillary layer in groups rather than the usual pattern of distribution which was mostly scattered particularly at 2nd months of age.

S100 & MHC-II-both immune markers were used to identify APC cells of the skin. APC were expressed against both the antibodies. S100 positive cells were mostly found at the dermoepidermal junction and few in the epidermis also (Fig. 3), which revealed the typical dendritic cell and langerhans cells of the skin respectively. Morphologically the cell body was larger and processes were very short. The cells were identified from the date birth till the end of experimental age groups and numbers of cells were mostly constant in all the experimental age groups. There was no marked difference were identified in morphology and distribution of S100 positive cells in the skin. MHC-II positive cells revealed in the epidermis only and cells were mostly spindle shaped with

small cytoplasmic process. MHC-II cells represented the langerhans cells of the skin and their frequency increased according to advancement of age, high frequency record from 3rd months onwards (Fig. 4).

Electron microscopy

The scanning electron microscopy picture of the skin of day old goat revealed numerous distributions of hairs present in groups emerging from stratum corneum. The numerous flaky appeared structures revealed the cells of stratum corneum (Fig. 5). The ultra-structure of skin (TEM) revealed the cells of the stratum basale with huge distribution of cells organelles. The ultra-structure of skin revealed the dermal component of the skin with huge distribution of connective tissue fibre. The arrangement of fibre in different directions revealed normal fibre distribution within dermal component. However in the limited sample langerhans cell was not identified Ultra structurally (Fig. 6).

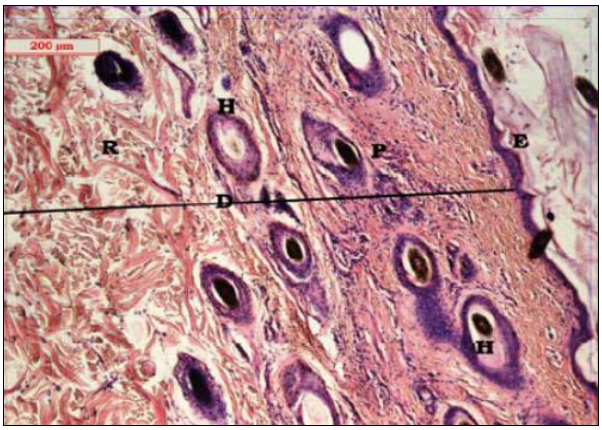

Fig 1: Skin (H&E) -1st Month

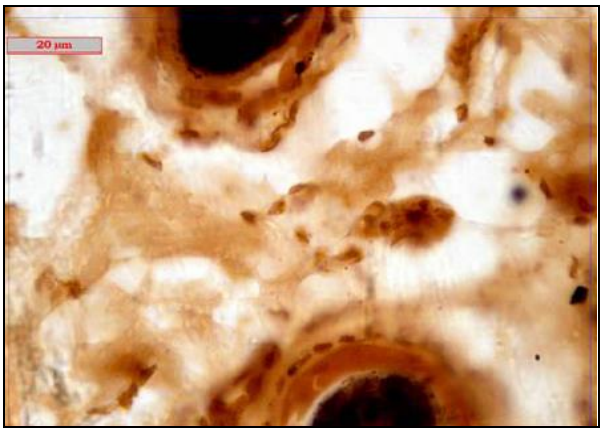

Fig 2: Skin (CD57) – Day old

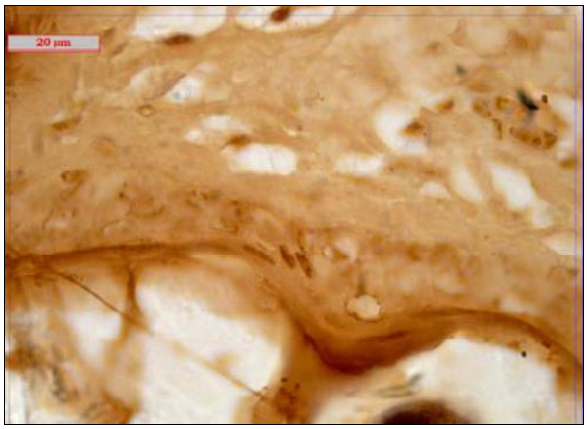

Fig 3: Skin (S100) – 5th Months

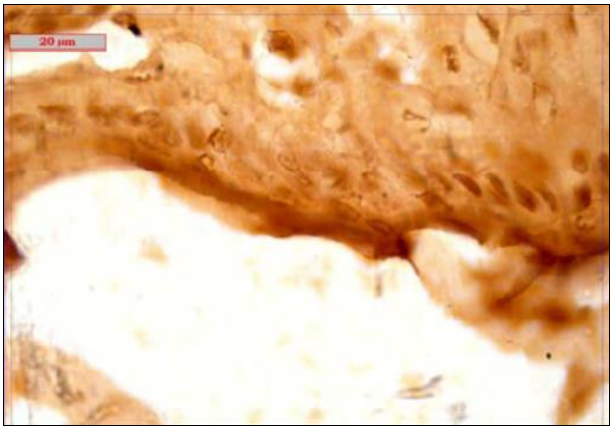

Fig 4: Skin (MHC II) – 2nd Months

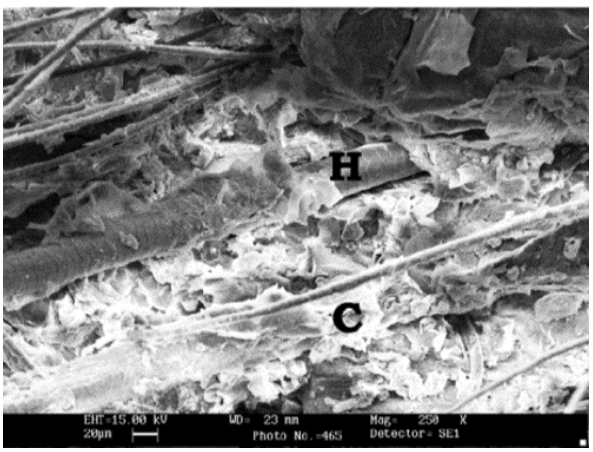

Fig 5: Skin (SEM) – Day old

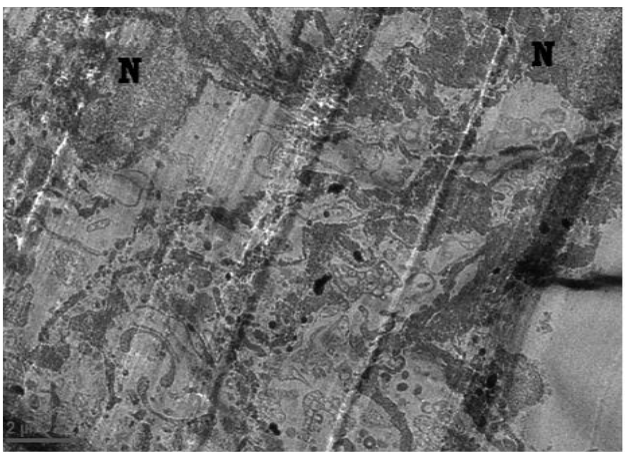

Fig 6: Skin (TEM) – Day old

Fig 1: Photomicrographs showing the histoarchitecture of skin. E= Epithelium, D= Dermis, H= Hair follicle, R= Reticular dermis, P= dermal papilla. Fig. 2-4. Showing the immunohistochemistry of skin in different age groups. Fig. 2 showing CD57 positive cells, Fig. 3 showing S100 positive cells, Fig. 4. Showing MHC II positive cells, Fig. 5. Scanning Electron Micrographs showing depicting hairs (H) occurring in groups from the stratum corneum (C). Fig.6. Transmission electron micrographs showing nucleus (N) and organelles in the skin.

Discussion

Histological study

In the present study the skin samples taken from the neck region revealed that epithelium with four layers from 1st month of age. Stratum lucidum was absent in all age groups. Stratum corneum was found absent at birth. The epidermis was lined by keratinized stratified squamous epithelium. Similar observation was recorded by Ahmad *et al*. (2010) [1] in case of the skin of the Madras red sheep. The skin thickness increased gradually with the increment of age. The depth of the sweat gland, hair follicle was increased with age. This is in accordance with Ahmad *et al*. (2010) [1] in case of sheep. The presence of keratinocytes was from 1st month onwards responsible for giving innate immunity by creating antibacterial activity.

Immunohistochemistry

CD57 marker expressed against caprine NK cells which were mostly found from date of birth. But no literature was found in support of our present study. S100 and MHC II markers were used to identify dendritic cells of the skin. Bautista at al. (2002) [2] expressed high levels of MHC class II antigen. S100 polyclonal antibody marked DC in the dermoepidermal junction and few in the epidermis. Morphologically cell body was large and processes were shorter. Oliveira-Sequeira *et al*. (2000) [5] also identified DC by S100 antigen. Oliveira-Sequeira *et al*. (2000) [5] defined S100 positive morphology which differed from our present findings. Whereas MHC II antigen identified only langerhans' cells of the epidermis which is partly in agreement with Nestle *et al*. (1998) [4].

Conclusion

From the present study it may be concluded that skin as a whole the largest organ of the body gives the natural immunity from the date of birth. Stratum lucidum was absent in all age groups. The skin thickness increased gradually with the increment of age. The depth of the sweat gland, hair follicle was increased with age. The presence of different APC and NK cells and there increment with age revealed the active role of skin to combat the body defense mechanism from date of birth. But initially up to 2nd months there was some less population but from 3rd months the number of APC and NK cells increased rapidly.

Acknowledgments

We acknowledge the financial support rendered by the authority of West Bengal University of Animal and Fishery Sciences Kolkata for undertaking the research project.

References

1. Ahmad MS, Sathyammoorthy OR, Ramesh G. Regional variations in the microscopic structure of skin in postnatal Madras Red sheep (*Ovis aries*). Ind. J of vet. Anatomy. 2010; 22(2):33-39.
2. Bautista EM, Gregg D, Golde WT. Characterization and functional analysis of skin-derived dendritic cells from swin without a requirement for *in vitro* propagation. Vet. Immunol. Immunopathology. 2002; 88:131-148.
3. Luna LG. Manual of histologic staining method of the Armed Force Institute of pathology. 3rd Edn. Mac. Graw-Hill Book Company, Ny, 1968
4. Nestle FO, Filgueira L, Nickoloff BJ, Burg G. Human dermal dendritic cells process and present soluble protein antigens. J Invest. Dermatol. 1998; 110(5):762-6.
5. Oliveira-Sequeira TCG, Bacchi CE, Lello E. S-100 dendritic cells in normal and Dermatobia hominis infested cattle skin. Brazilian Journal of Veterinary Research and Animal Science. 2000; 37(4):299-303.
6. Tizard IR, Chubot RM. Veterinary Immunology: An Introduction, 6th ed. WB Saunders, Philadelphia, 2000, 480.