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Ultrastructural studies on the skin of Vechur cattle of Kerala

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Abstract
Ultrastructural studies were conducted on the skin of Vechur cattle of Kerala. Skin samples of 1 cm^2 size were collected from 15 different regions *viz*., dorsal, ventral and lateral regions of head, neck, abdomen and tail respectively and muzzle, interdigital region of fore and hind limbs. For Scanning Electron Microscopy (SEM) samples of skin were fixed in 2.5 percent gluteraldehyde in 0.1M phosphate buffer (PBS) (pH 7.2) for 12 h at room temperature and post fixed in one percent aqueous osmium tetroxide for two hours at 4 ºC followed by 1X PBS washing twice for 30 min each as described by Shapiro *et al*. (2019) in rabbits. After standard procedures, the samples were subjected to electron beams for Visualization under monitor at accelerating voltage of 20kV. In scanning electron microscopy, a set of rectangular-shaped deep channels were observed on the epidermal surface. In the muzzle area, hexagonal-shaped raised regions were separated by narrow, deep grooves. Oval-shaped openings of the nasolabial glands were visible on the muzzle's surface, with irregular borders caused by numerous sulci. Within the stratum corneum, there were four to five layers of well-defined, scale-like flattened keratinised dead cells. Dermal papillae were seen as upward protrusions in the papillary dermis. A significant number of collagen fibres ran parallel to the skin's surface, with some exhibiting a longitudinal and concentric arrangement around the sweat glands, sebaceous glands, and hair follicles. Elastic fibres formed a sponge-like elastic system in the papillary dermis. The collagen fibre bundles in the reticular dermis were thicker in comparison to those in the papillary dermis. The hair follicles in dermis were confined to the upper third or fourth of the thick dermis. Numerous delicate elastic fibres formed a network and enveloped the hair bulbs located in the lower part of the follicles. Pairs of lobulated sebaceous glands were observed in the reticular layer of the dermis.

Keywords: Ultrastructural studies, skin, Vechur cattle, Kerala

Introduction
Among the native cattle breeds in Kerala, the Vechur is a dwarf cattle and is regarded as the world's smallest breed of cattle. The Vechur cows are noted for its increased disease resistance and heat tolerance, when compared to the crossbred cattle. Vechur cows are one of the top milk producers when compared to other dwarf cattle breeds since they are acclimated to the hot, humid climate of Kerala.

Skin, constituting about 15 percent of the total adult body weight, serves as the body's largest organ and plays a crucial role in communication with the environment. Skin structure varies considerably across species, and these anatomical and morphological distinctions significantly contribute to an animal's ability to withstand heat stress. Literature pertaining to the characteristics of skin in Vechur cattle is scanty and hence the present work was undertaken.

Materials and Methods
For Scanning Electron Microscopy (SEM) samples of skin were fixed in 2.5 percent gluteraldehyde in 0.1M phosphate buffer (PBS) (pH 7.2) for 12 h at room temperature and post fixed in one percent aqueous osmium tetroxide for two hours at 4 ºC followed by 1X PBS washing twice for 30 min each. Later the samples were dehydrated with graded ethanol (20 percent, 40 percent, 60 percent, 80 percent and 100 percent) at 30 min each interval and were subjected to critical point dehydration for upto 3 h to reach a critical point carbon-di-oxide (CO_2). Thereafter CO_2 was bled out and samples were placed on the stubs and subjected to gold sputter for 90 seconds so that the gold particles get sputtered over the samples for better

visualization under electron beams. The samples along with stub were then placed in the scanning electron microscopy chamber (Ziess EVO 18 SEM. Germany) and vacuum was created and the working distance was adjusted manually. The samples were then subjected to electron beams for visualization under monitor at accelerating voltage of 20kV. Upon obtaining the images, the pore size was measured (n=5 each) using 'Image J' software.

Results and Discussion

Epidermis: On the epidermal surface, a set of rectangular-shaped deep channels was observed (Fig. 1). In the muzzle area, hexagonal-shaped raised regions were separated by narrow, deep grooves. These raised areas contained numerous microplicae and micropits, divided by septae, giving them a honeycomb-like appearance. The shape of the elevated regions on the nasal skin differed based on the animal species. In buffalo, cattle, small ruminant, cat, dog and fox, these patterns took the form of quadrilateral, hexagonal, square, cobblestone-like elevations, button-like structures with central depressions, and squamous hexagonal-shaped patterns with concentric layers respectively [1].

Oval-shaped openings of the nasolabial glands were visible on the muzzle's surface, with irregular borders caused by numerous sulci (Fig. 2). Similar observations in the muzzle of buffalo and goats were made [1]. However, the shape of the openings of these glands were whirlpool-shaped in goat and ovoid-shaped embedded within the nasal skin in sheep.

The skin around the nostrils of animals of the same species remained consistent over the course of an animal's life and was also utilized for identification through the application of nose prints [2]. The dampness of the nasal skin was a result of the secretions produced by the glands beneath the surface in cattle and pigs, while in dogs, it was primarily due to the secretions from the nasal mucosa, particularly the lateral nasal glands [3].

As reported earlier in domestic mammals, in the stratum corneum, in the present study there were four to five layers of well-defined, scale-like flattened keratinised dead cells. The outermost layer, known as the stratum disjunctum, showed an abundance of desquamated cell flakes [4]. Epidermal/rete pegs were clearly distinguishable in the skin of the muzzle (Fig. 3). The stratum corneum of epidermis, had dead cells and detached continuously in rats [5].

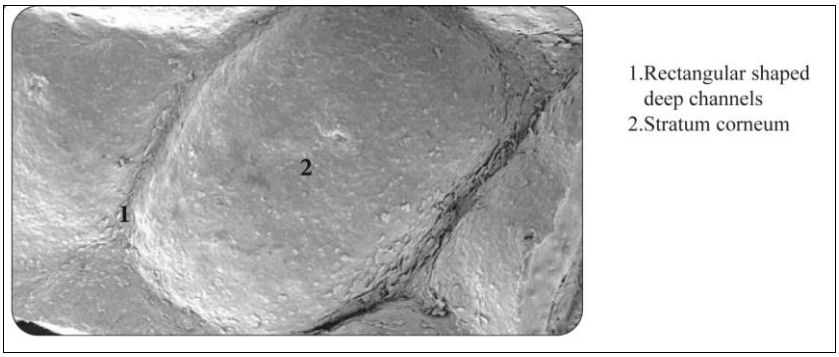

Fig 1: Skin of muzzle showing rectangular-shaped deep channels in Vechur cattle. SEM x 211

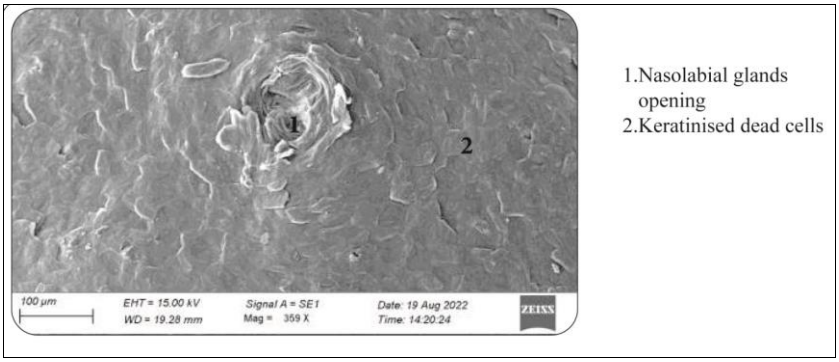

Fig 2: Skin of muzzle showing nasolabial glands in Vechur cattle. SEM x 359

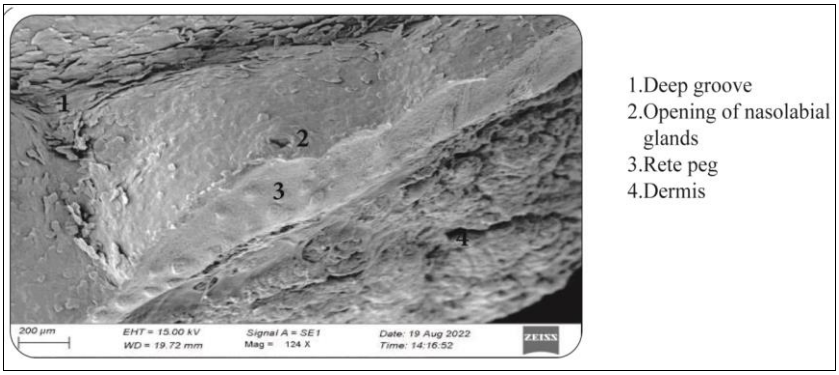

Fig 3: Skin of muzzle in Vechur cattle showing rete pegs. SEM x 124

Dermis

In both the cattle breeds dermal papillae were seen as upward protrusions in the papillary layer of the dermis (Fig. 4). The dermal papillae and rete pegs significantly augmented the surface area between the dermis and epidermis [6]. Since the primary role of the dermis was to provide support to the epidermis, this enlarged contact area substantially enhanced the exchange of oxygen, nutrients, and waste products between these two layers. Moreover, the expanded surface area reinforced the connection between the dermal and epidermal layers, effectively preventing their detachment from each other [7].

A significant number of collagen fibres ran parallel to the skin's surface, with some exhibiting a longitudinal and concentric arrangement around the sweat glands, sebaceous glands, and hair follicles (Fig. 5). The collagen fibre bundles in the reticular dermis were thicker in comparison to those in the papillary dermis. These closely interwoven collagen fibres provided the dermis with the ability to withstand high stress, as described earlier [8]. Elastic fibres formed a sponge-like elastic system in the papillary dermis, appearing as bundles of branching fibres. The characteristic feature of skin in maintaining constant tension was due to the presence of a wide-meshed sponge of elastic fibres that extended throughout the entire dermis [9]. The collagen served as the predominant structural element in the dermis [10]. Dermis exceeded the epidermis in thickness, offered support to the epidermis and contributed to the skin's elasticity due to its composition of elastic and collagen fibres [11].

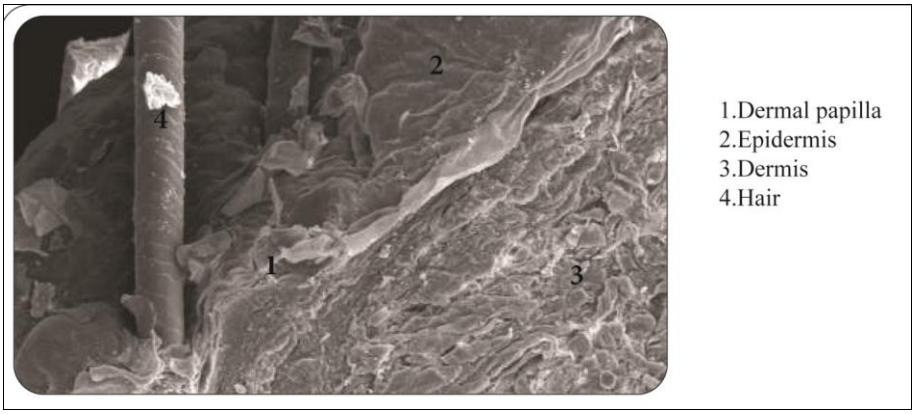

Fig 4: Skin of interdigital hindlimb showing dermal papilla in Vechur cattle. SEM x 363

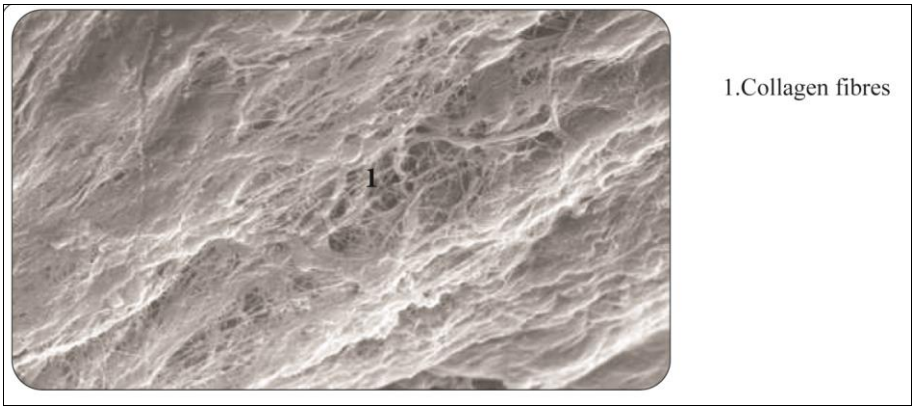

Fig 5: Skin of abdomen in Vechur cattle showing collagen fibres. SEM x 4.49 K

Hair and Hair Follicles

The dermis was regularly penetrated by large primary hair follicles (Fig. 6). These hair follicles were confined to the upper third or fourth of the thick dermis and had a cylindrical shape. Numerous delicate elastic fibres formed a network and enveloped the hair bulbs located in the lower part of the follicles (Fig. 7), as reported earlier in domestic mammals [4]. The surface of the hair was surrounded by overlapping scale-like cells. The scale margins in the hair were closely aligned and did not protrude from the hair shaft. Well-defined transverse waves of scales with smooth edges were observed at regular intervals. There was close interaction between the cells of the hair cuticle and the inner root sheath. In the case of tactile hairs, the external dermal sheath was notably closer to the hair follicles, and in the most distal area of the hair follicle, the inner and external dermal sheaths were in close proximity to each other. Scanning electron microscopic studies earlier also revealed that scale like cells covered the hair surface [4].

Each hair was encircled by three to four concentric keratinised layers, with a ring of cells that resembled the petals of a flower enveloping them [12].

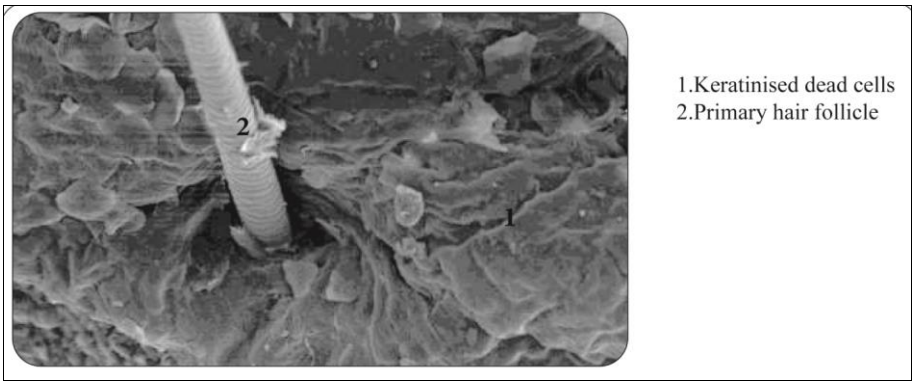

Fig 6: Skin of dorsal abdomen in Vechur cattle showing large primary hair follicles. SEM x 808

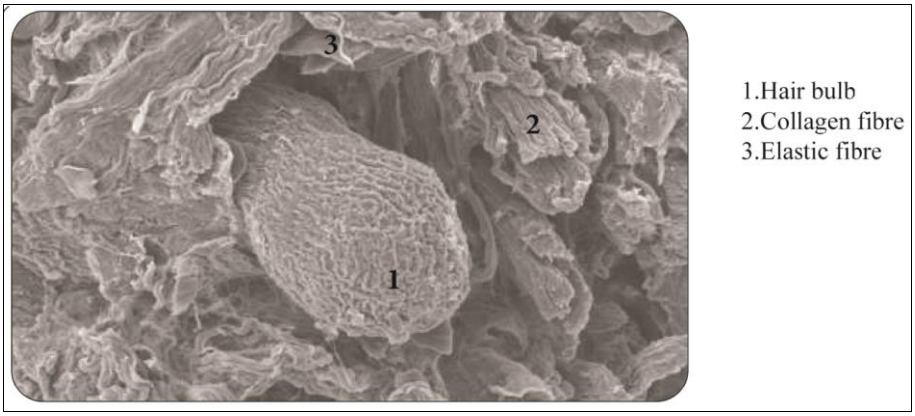

Fig 7: Skin of dorsal abdomen showing elastic fibres around the hair bulbs in Vechur cattle. SEM x 604

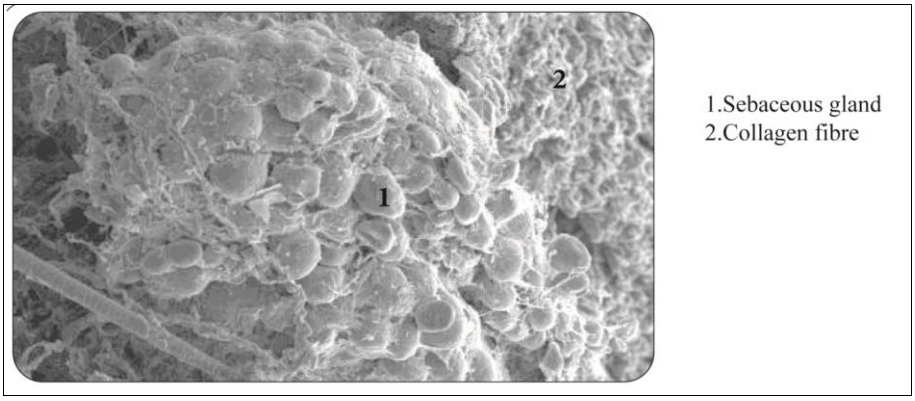

Fig 8: Skin of dorsal abdomen showing sebaceous glands in Vechur cattle. SEM x 325

Arrectoris Pilorum Muscles

Similar to the earlier reports, in the present study, the smooth muscle arrector pili was placed obliquely upwards from the base of the bulged region of hair follicle to the upper dermis [8]. The muscle was seen encircling the hair follicle. A close connection of this muscle with elastic fibres was seen near the attachment to the hair follicle and the undersurface of the epidermis.

Sebaceous Glands

As reported earlier in domestic mammals [8] and in sheep, goat and deer [10], pairs of lobulated sebaceous glands were observed in the reticular layer of the dermis. These sebaceous glands were enveloped by a connective tissue sheath primarily composed of collagenous fibres and fibroblasts (Fig. 8).

Summary and Conclusions

In scanning electron microscopy, a set of rectangular-shaped deep channels were observed on the epidermal surface. In the muzzle area, hexagonal-shaped raised regions were separated by narrow, deep grooves. These raised areas contained numerous microplicae and micropits, divided by septae, giving them a honeycomb-like appearance. Oval-shaped openings of the nasolabial glands were visible on the muzzle's surface, with irregular borders caused by numerous sulci. Within the stratum corneum, there were four to five layers of well-defined, scale-like flattened keratinised dead cells. The outermost layer, known as the stratum disjunctum, showed an abundance of desquamated cell flakes. Epidermal pegs were clearly distinguishable in the skin of the muzzle. Dermal papillae were seen as upward protrusions in the papillary dermis. A significant number of collagen fibres ran parallel to the skin's surface, with some exhibiting a longitudinal and concentric arrangement around the sweat glands, sebaceous glands, and hair follicles. Elastic fibres formed a sponge-like elastic system in the papillary dermis, appearing as bundles of branching fibres. The collagen fibre bundles in the reticular dermis were thicker in comparison to those in the papillary dermis. The dermis was regularly penetrated by large primary hair follicles. These hair follicles were confined to the upper

third or fourth of the thick dermis and had a cylindrical shape. Numerous delicate elastic fibres formed a network and enveloped the hair bulbs located in the lower part of the follicles. The surface of the hair was surrounded by overlapping scale-like cells. The scale margins in the hair were closely aligned and did not protrude from the hair shaft. Well-defined transverse waves of scales with smooth edges were observed at regular intervals. There was close interaction between the cells of the hair cuticle and the inner root sheath. In the case of tactile hairs, the external dermal sheath was notably closer to the hair follicles, and in the most distal area of the hair follicle, the inner and external dermal sheaths were in close proximity to each other. The smooth muscle arrector pili was placed obliquely upwards from the base of the bulged region of hair follicle to the upper dermis. The muscle was seen encircling the hair follicle. A close connection of this muscle with elastic fibres was seen near the attachment to the hair follicle and the undersurface of the epidermis. Pairs of lobulated sebaceous glands were observed in the reticular layer of the dermis. These sebaceous glands were enveloped by a connective tissue sheath primarily composed of collagenous fibres and fibroblasts.

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