HISTOLOGICAL STUDIES ON ANTLER OF SPOTTED DEER (*Axis axis*) AND HORNs OF BLACK BUCK (*Antelope cervicapra*) WITH THAT OF HORNs OF DOMESTIC CATTLE

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Abstract: The present study was conducted on antlers of spotted deer and on horns of black buck and cattle with the objective of differentiating them from each other. In the horizontal ground section of antler four distinct zones were observed namely subvelvet zone, zone of osteonic bone, transition zone and spongiosa zone from periphery to the center of the antler. Similar type of zonation was also observed at the tip of the tine of antler. Such zonations were not observed either in the transverse ground section of burr and pedicle of the antler, as well as in Cattle and Black buck horn core. The transverse section of the soft tissue located between the horn sheath and horn core of cattle showed stratified squamous epithelium towards horn sheath. Underlying this epithelial layer was a thin layer of highly vascularised loosely arranged dermis composed of irregularly oriented collagen fibres.

Key words: Antlers; Horns, *Axis axis*, *Antelope cervicapra*

INTRODUCTION

Antlers are unique to cervids and found mostly on males; only caribou and reindeer females have antlers, which were normally smaller than those of the males. A horn is a pointed projection of skin on the head of various animals consisting keratin covering and other proteins around the core of live bone. True horns are found mainly among the ruminant artiodactyls, such as in the families of Antilocapridae (pranghorn) and Bovidae (cattle, goats, antelope etc).

Antlers have been used by craftspeople since ancient times to make tools, weapons, ornaments and toys [1]. In the velvet stage deer have been used in Asia as a dietary supplement or alternative medicinal substance for more than 2,000 years [2]. Deer antler is also used to make “Horn buttons”. Black buck horns were sometimes hunted to display as a hunting trophy or as a decorative object. This practice has been declared as illegal and dangerous as black buck were considered near threatened by IUCN due to reduced population partially from pressure of such hunting. Black buck horns, horn tips and spotted deer antler tips were also used for centuries in the manufacture of scales, grips or handles of knives and other weapons, handle scales of hand guns.

Proper identification of these artifacts is very important, since various ceramic and biological materials are often used as substitute materials. The present study was conducted to identify and differentiate the antlers of spotted deer, horns of black buck and horns of cattle by using histological technique in order to facilitate solving veterolegal cases. The identification of the correct specimen is of utmost importance from the point of punishing the poachers and also in the conservation of these wild animals.
MATERIALS AND METHODS

For histological study antler and horn cores were cut into thin cross sections using bone saw cutter. For histological study ground sectioning method was followed.

Ground sectioning: Cross sections of antlers and horn cores were rubbed against Carborandum stone manually until the sections became very thin for light to pass through. Such sections were observed under light microscope and observations were recorded. The ground sections were stained with H & E Phloxine [3]. Such sections were observed under light microscope and observations were recorded.

Connective tissue between the horn sheath and horn core were cut into 1 cm² size and fixed in 10% neutral buffered formalin for a minimum of 48 hours. The samples were processed for paraffine embedding and 5 µm thick transverse sections were cut. The transverse sections were stained with H and E Phloxine [3] and Masson’s Trichrome [4]. Microscopic photos were taken using Olympus -CX41 microscope attached with Digital Microscope Camera ProgRes® C3.

RESULTS

1. ANTLERS OF SPOTTED DEER

Antler consisted of beam, tine, burr and pedicle. The samples were taken from these regions.

A. Beam: The cross section of antler beam showed typical arrangement of different zones which can be subdivided from the periphery to the centre of the antler as follows:

i) Sub velvet zone: The sub velvet zone presented a thin homogenous layer of osteonic bone tissue with large number of blood vessels and haversian canals running parallel to the peripheral layer. Lack of mineralisation within these subvelvet zone appeared as thin homogenous mass (Fig. 1).

ii) Zone of osteonic bone: This zone appeared as a compact lamellar bone surrounding the substantia spongiosa. Only small haversian canals and interstitial lamellae were seen (Fig. 1).

iii) Transition zone: There was a gradual change from the zone of osteonic bone to trabacular bone.

iv) Spongiosa: The spongiosa consisted of thick strip of trabacular bone having spongy trabaculae which lacked lacunae and osteoid seams but however contained masses of tissue resembling the marrow substance (Fig. 3).

B. Tine tip: Four zones were observed in the tine tip also. However the zone of osteonic bone showed small osteoid seams (haversian system). Haversian canals were well developed with large and intercommunicating Volkmann’s canals. Lamellae surrounding the osteoblast cells were well developed with few areas of mineralisation.

The transition zone presented a gradual transition from the zone of osteonic bone to spongiosa zone enclosing large trabaculae containing blood vessels. The blood vessels were surrounded by complete lamellar system lacking haversian system. The spongiosa zone was a thin rim from which large number of blood vessels projected towards the transition zone. It was a homogenous mass. There were few osteoid seams.

C. Burr: At the periphery of the burr there was a homogenous layer of periosteum with perforating blood vessels running towards the deeper part. Large number of blood vessels were traversing into the deeper part of the burr region enclosed in haversian system. The haversian system were irregular in shape exhibiting interstitial lamellae (Fig. 4). Large numbers of perforating canals were seen interconnecting haversian canals. Towards the centre of the burr haversian system were reduced in number and towards the inner most part there was more resorption of bone (Fig. 5). Multinucleated giant cells called osteoclast were seen towards the centre of the burr participating in the bone resorption process.

D. Pedicle: The peripheral area of pedicle showed a homogenous rim made of bundles of collagen fibres (sharpey’s fibres) connecting the periosteal layer to the bone. Below the fibrous layer, well developed haversian systems with interstitial lamellae were present (Fig. 6). The haversian canals were smaller in diameter, however their size increased towards the centre of the pedicle. In addition at places there
Fig. 1: Transverse section at the beam of the spotted deer antler showing subvelvet zone (SV) and osteonic zone (OZ). Ground section x 40.

Fig. 2: Transverse section at the beam of the spotted deer antler showing transition zone. Ground section x 40.

Fig. 3: Transverse section at the beam of the spotted deer antler showing spongiosa zone. Ground section x 40.

Fig. 4: Transverse section at the burr of the spotted deer antler showing haversian system in irregular shape. Ground section x 40.

Fig. 5: Transverse section at the burr of the spotted deer antler showing reduced number of haversian systems and areas of bone resorption (arrows). Ground section x 40.
was resorption of the osteon by the activity of osteoclast cells. The canalicular system enclosing the osteoblast lacunae were well defined surrounding the haversian canals, and were spindle shaped, arranged perpendicularly in different haversian lamellae surrounding the haversian canal and blood vessels lying in the centre of the pedicle.

2. BLACK BUCK HORN

**Base:** The base of the horn core presented homogenous irregular surface rich in collagen fibres resembling the periosteal bone. Just below the periostium there were large numbers of haversian system representing well developed lamellae lined with osteoblast cells. The Haversian canals were interconnected by large number of perforating canals (Fig. 7). In between there were large spaces which were occupied by homogenous mass representing the pulp of the core (Fig. 8). The peripheral boundaries of the empty spaces were surrounded by haversian lamellae having osteocytes. The lacunae were shrunken with osteocytes. Peripheral lacunae were occupied by well developed osteoblast with well defined canaliculi.

**Tip:** The tip of the horn resembled that of the base except that there were increased number and sizes of hollow spaces forming the pulp of the horn core. The vascular channels were more indicative of nourishment to the growing horn. Towards the centre of the tip was also represented by well developed osteoblast cells and numbers of osteocytes were less compared to that observed at the base.

3. CATTLE HORN:

The horn core presented towards the periphery a homogenous periosteal layer. Below this there were blood vessels along with well developed haversian system (Fig. 9). The inner part of the horn haversian system was not seen. However, there was increased amount of vascularity. The blood vessels were running circularly around the core of the horn. Large empty spaces were present towards the inner surface of horn core lined by collagen fibers.

The transverse section of the soft tissue located between the horn sheath and horn core showed stratified squamous epithelium (towards horn sheath). Below this epithelial layer, a thin layer of highly vascularised loosely arranged dermis composed of irregularly oriented collagen fibers with stray fat cells and a sparsely distributed connective tissue cells around the blood vessels were noticed in Masson’s trichrome stain (Fig. 10). Further connective tissue of the dermis formed papillae by projecting amongst the epithelium. Underlying this was the dense irregular connective tissue of the hypodermis.

**DISCUSSION**

The present study was conducted on antler of spotted deer and horns of black buck which are most commonly poached wild animals for trophy making using their head gear. They belong to the order Artiodactyla in the animal kingdom with the spotted deer belonging to the family cervidae while the black buck belongs to family bovidae. The study also attempted to compare antler of spotted deer and horn of Black buck and cattle.

In the present study cross section of spotted deer antler showed four clear zones. This observation corroborated with the observation of zonations in cross section of fallow deer antler described four zones, from outside to inside [5]. But in cross sections of burr and pedicle of spotted deer antler, such typical zonations were not observed in the present study. Further, in cattle and black buck horn core such zones were not observed. Whereas, the antler has an inner porous structure similar to spongy bone surrounded by a very solid compact bone, as in mammalian long bone and the haversian or osteonal structures in antler consist only of primary osteons [6]. Secondary osteons do not occur in antler bone since growth is fast i.e. usually three to four months, after which there is no blood supply and no reconstruction unlike normal mammalian long bone

The sub velvet zone observed in antler presented a thin homogenous layer with osteonic bone tissue with large number of blood vessels and haversian canals running parallel to the peripheral layer. Lack of mineralisation within these subvelvet zone appeared as thin homogenous mass. This observation concurred with the observations made in fallow deer antler and further lack of mineralization within these osteoid seams was the main reason why the subvelvet zone appear as a thin spongy area confined to the periphery of the antler shaft [5].

The zone of osteonic bone was a compact lamellar bone surrounding the substancia spongiosa. Only small haversian canals and interstitial lamellae were observed. The small osteoid seams was detected
Fig. 6: Transverse section at the pedicle of the spotted deer antler showing well developed haversian systems at outer zone. Ground section x100

Fig. 7: Transverse section of horn core of black buck horn showing large number of interconnecting perforating canals. Ground section x40

Fig. 8: Transverse section of horn core of black buck horn showing inner zone. Ground section x40

Fig. 9: Transverse section of cattle horn core showing large numbers of blood vessels (BV) along with well developed haversian system (HV). Ground section x40

Fig. 10: Transverse section of soft tissue located between the horn sheath and horn core showing a thin layer of stratified epithelium (SE), thick layer of dermis (DM) and blood vessels (BV). Connective tissue of the dermis forming papillae by projecting amongst the epithelium (arrow). Masson’s Trichrome stain x100
within zone of osteonic bone and osteones consisting of concentric and interstitial lamellae were mainly orientated in the long axis of the deer antler shaft [5].

The transition zone exhibited a gradual transition from osteonic bone to trabacular bone (inner spongiosa) which corroborated with the observation made in fallow deer antler. However in the transition zone from the periphery to the center, haversian canals of increasing sizes as well as osteoid seams of increased thickness were observed [5].

In the present study the spongiosa zone of antler consisted of thick strip of trabacular bone having spongy trabaculae which lacked lacunae and osteoid seams but contained masses of tissue resembling the marrow substances were found. Towards the centre antlers have cancellous (spongy) bone but unlike other spong bone in the body, such as sternum and pelvis, the bone of the antler does not manufacture blood, although there is considerable blood and a little fatty marrow in it [7]. In fallow deer antler spongiosa zone was described as the area of trabecular bone which was a less dense cancellous bone consisting of spongy trabaculae and spacious lacunae [5]. The trabeculae are covered with osteoid seams of varying thickness similar to the findings of the present study.

In the microscopic observation of antler just after velvet shedding exhibited a widespread capillary system within the cortical layers sub velvet zone, zone of osteonic bone and transition zone as well as in the areas resembling bone marrow within the spongiosa [5]. The capillary system also leads directly to the outer border of the antler. However, the capillary system was not clearly appreciable in the above zones in the present study.

After the loss of soft tissue the vessels occupying the canal in the superficial dense layer of bone dries up, while those in the central spongiosa continue to receive blood but only for a limited period. Once freed of its velvet and lost its blood supply the antler is no longer a viable structure and hence sheds annually. After shedding of the antler the new growth occurs from pedicle which remains on the surface of the frontal bone and gradually the antler develops by the genetically determined tines and ossifies over which a new velvet covered structure grows [8].

The pedicle bone is divided into outer fibrous and inner cellular layers and observed that osteoblast were arranged more vertically separated by highly vascularized mesenchymal tissue [9]. However in the present study, below the fibrous layer osteoblastic activities with the formation of spongiosa layer were seen having intracartilagenous ossification with a well developed haversian system.

The soft tissue located between the horn sheath and horn core showed outer portion (towards horn sheath) lined with stratified squamous epithelium and underlying the epithelium was a corium layer. In general the epithelium layer produces keratin which forms the horn sheath but the horn corium was a vascular and innervated that supplies blood and nutrients to the germinal cells that create the keratinized stratum corneum of the horn [10]. Compared to cattle horn, the soft tissue located between the horn sheath and horn core of black buck was very thin and was membrane-like and could not be separated from the horn core for further processing.

To conclude in brief, identifying and differentiating horns and antlers, histologically antler can be easily differentiated from horn core because of the clear visibility of zonations in cross section, which cannot be observed in the horn core. This study will help to solve the veterinary cases.

REFERENCES