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Identification of acinar cells of salivary gland in blood fed female ticks (*Hyalomma anatolicusm anatolicum*) by light microscopy

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ABSTRACT

Ticks play an important role in human and veterinary medicine particularly due to their ability to transmit protozoan pathogens. This study was undertaken on salivary gland of tick using histological methods to decrease cost and budget to determine the presence of tick-borne pathogens of medical and veterinary importance. Ticks have been proved as carrier or vector of pathogenic protozoa by separating salivary gland and using histological methods. This study provides the morphological and histological properties of the salivary glands of semi-engorged *Hyalomma anatolicum anatolicum* females. Unfed ticks solely were placed on cattle's ear for feeding and females were collected, and placed in glass vials containing 70% ethanol. Collected ticks were studied and identified morphologically. Dorsal exoskeleton removed with a scalpel and salivary glands were separated by suitable forceps. Then Salivary glands were fixed in 10% formalin for further studies by light microscopy. Samples were stained with hematoxylin-eosin (H&E) for investigation under light microscope. The histological results show that the glandular tissue in females is combined with a system of ducts and the salivary glands of *H. a. anatolicum* consisted of three types of acinus (acinus I, II and III). The type I acinus was agranular and showed slight morphological changes during feeding. There were five granular cell types in the type II acinus, and three granular cell types in type III acinus. Data achieved here will help in understanding of the cellular morphology and general histology of these organs in this specie, preparing important information for the creation of scientific bases which will contribute to the development of more specific and efficient methods of control.

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Introduction

There are three types of salivary gland acini in ixodidae female ticks (Nunes et al, 2006; Hall-Mendelin et al, 2011; Šimo et al, 2017; Goddard & Varela-Stokes., 2009; Bior et al, 2002; El-Kady et al, 2001). Type I acinus are directly attached to the anterior region of the main salivary duct. The type I acinar cells appear as the cells with large nuclei. The numbers of cell, the shapes of the cells as well as granularity does not change during the period of feeding (Zhou et al, 2013; Bowman & Sauer, 2005). Type II acinus connects to lobular ducts. This type consists of three cell types; a, b and c cell (Šimo et al, 2017; Bowman & Sauer, 2005). Type III acinus is occupied posterior part of the lobulated mass of the gland. They are connected to

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the main salivary duct or to its branches by compound lobular ducts. Type III contains'd', 'e' and 'f' cell (Zhou et al, 2013; Godwin & Marius., 2017). Type II and III acini have been subjected to many studies due to their morphological changes during different stages of life cycle of the tick. In some studies of tick salivary glands, Haematoxyline and Eosin dye has been used for staining paraffin – embedded sections. Haematoxyline and Eosin (H and E) stain (Zhou et al, 2013) used in sections prepared from salivary gland of tick. (Bowman & Sauer, 2005). Tick salivary gland is important due to histology and from the practical point of view; the salivary gland is the place of living pathogenic blood protozoan agent. In recent years, the salivary gland of ticks has been the focus of study as a source of antigen to make a novel vaccine against blood feeding tick (Aeschliman 1990; Vancová, 2010). Therefore it was decided to study the anatomy and histo-morphology of salivary gland using two types of staining methods in blood fed ticks.

Materials and methods

Animals: *Hyalomma anatolicum* ticks were taken from research Laboratory dealing with Study of ticks and tick borne diseases. Ticks were separately bred in a specialized laboratory for tick and tick borne study. Native local bred sheep (Fashandi) serologically negative for *Theileria annulata* infections were used for adult tick feeding and white New Zealand rabbits were used for feeding of larvae and nymphs.

Ticks Identification: Different diagnostic characters of ticks have been regarded according to diagnostic keys presented by (Apanaskevich, 2003) using anatomical microscope and they have been confirmed as *Hyalomma anatolicum anatolicum* (Figure 1).

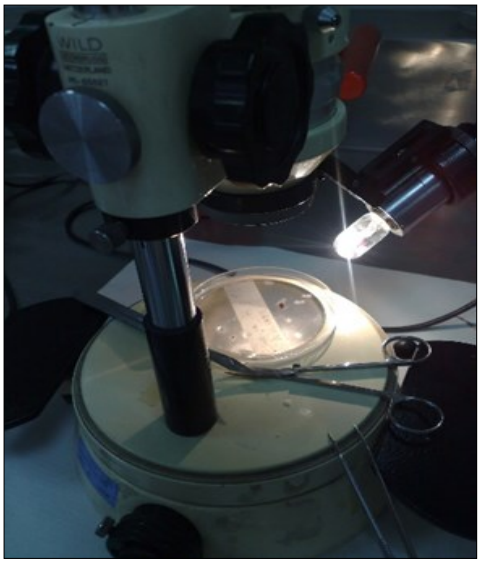

Figure 1. The ticks were examined under a stereomicroscope to identify.

Tick breeding: Adult ticks were placed on the ears of rabbits or on sheep. Engorged stages were kept at 28° C and 90-95% humidity for molting or oviposition. After that molting or hatching was completed the ticks were also kept at 20 °C and 80% relative humidity.

Histological processing: Immediately after removing ticks from the host, tegument of each tick was carefully perforated with fine needles and was then immersed in fixative solution (buffered formalin 10%, pH 7.0) for 12 h. After fixation, ticks were embedded in paraffin and processed according to routine histological techniques. Each sample was serially sectioned longitudinally at a thickness of 4 µm. Salivary glands were aimed as such as that incision place on the body of tick pass longitudinally or transversely from salivary gland in the tick body. The specimens were then dehydrated in a gradual series of ethanol and embedded in the paraffin. Each blocks were serially sectioned longitudinally at a thickness of 4 µm. Sections were stained with Haematoxyline and Eosin (H and E) and studied by light microscope.

Results

Morphology: The salivary glands of female ticks (*H.anatolicum*) are extended organs, situated laterally in the tick's body, and combined of rounded acini, from which arise the acinal ductules that collect the secretion made by the gland (Figure 2).

Figure 2. The salivary glands of females were placed

laterally in the tick's body.

Tubules having smaller diameter are attached to the common excretory duct, via the moderate ducts. The acini are presented with varying scales, perhaps because of feeding stage of tick and related secretory cycle in which the individual tick undergo (Figure 3).

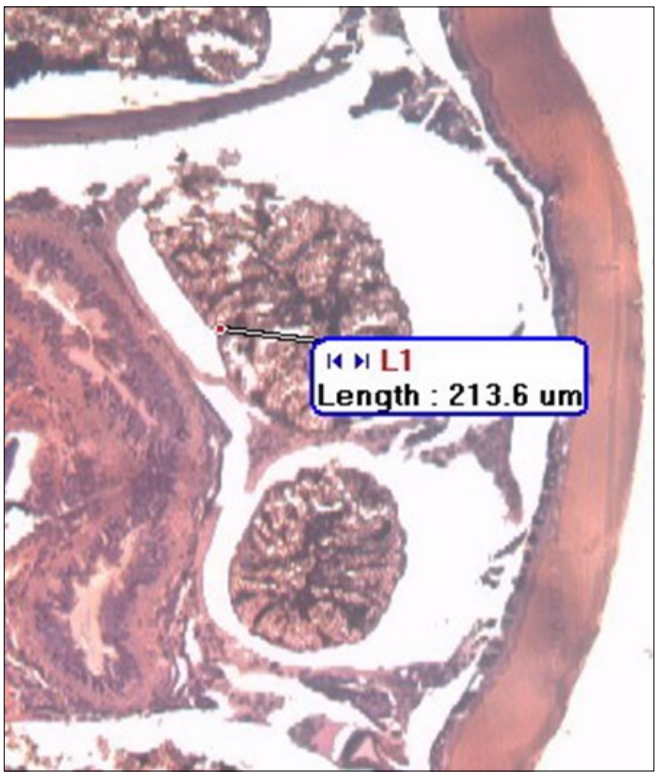

Figure 3. Histological sections of the salivary glands of semi-engorged *Myeloma anatolicum anatolicum* female ticks. (H&E×40).

Histological study

Type I acinus: The type I acini are agranular and located anteriorly in gland that directly is connected to the main duct through the lobular short canal. There are four different cell types in type I acini, including, peripheral or pyramidal, central, constrictor and neck or peritubular cells and they are classified based on Krolak et al. (1982) study. Round-shaped nuclei and a homogeneous cytoplasm slightly stained by eosin were seen. In central and peripheral cells the nuclei, being round-shaped and strongly stained by hematoxylin can also be observed (Figure 4).

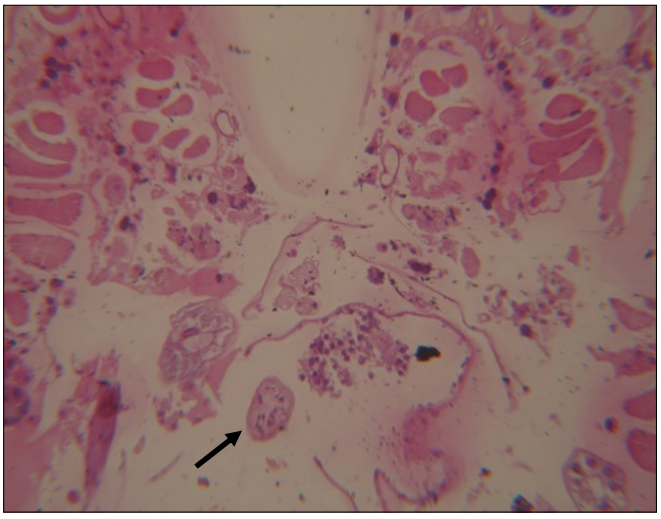

Figure 4. Salivary gland. Hyalomma anatolicum anatolicum female tick. Acini I. homogeneous cytoplasm and peripheral cells (arrow) were seen. (H&E×400).

Type II acinus: Type II acini contains five distinct kinds of granular cells (a-b-c1-c2-c3) that is arranged in a convergent status around a small duct. Those cells are separated with interstitial cells from each other. Branches are made from original duct of salivary gland and they are connected to each other via a cuticular acinar duct. There are three types of cells: one of them is (a-cell) that have secretion granules powerfully stained by eosin, while (b-c1-c3) are strongly stained by hematoxylin, and in c3 cells, large secretion granules are present, regarding to (b cells), the secretion granules are the largest studied granules for this type of acini and they are seen with different scales and are strongly stained with hematoxylin. According to C1 cells, secretion granules are strongly stained with hematoxylin. C2 cells are full of secretory granules which the background are stained both by eosin and hematoxylin (Figure 5).

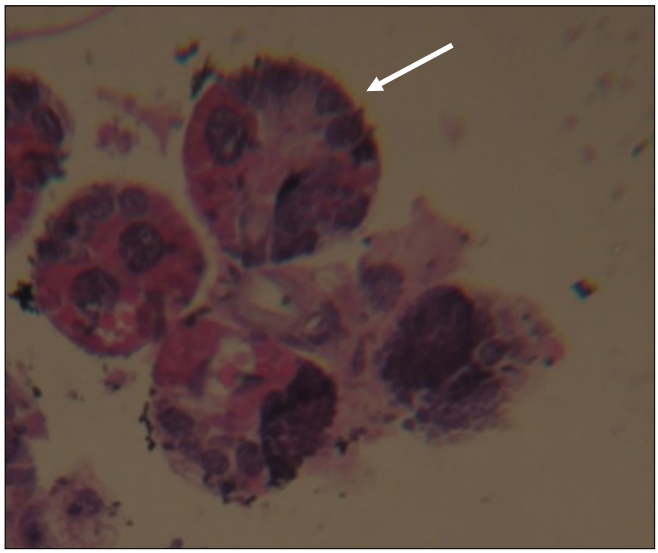

Figure 5. Salivary gland. *Hyalomma anatolicum anatolicum* female tick. Type II acini lumen with the original duct branches (arrow) were seen. (H&E×400).

Type III acinus: These acini are located in the distal area of the salivary glands. Each acinus contained of three granular cell types, (d, e and f) ordered around a collective lumen like type II acini. In unfed ticks the lumen of the acinus was seen to be small and contained a mass of microvilli compressed together along the center of the acinus. However, as feeding has progressed, the lumen enlarged and became more obvious. In unfed ticks the lumen of the acinus was tiny and included a mass of microvilli compacted together along the center of the acinus. Anyway, it is going to be more enlarged and become more apparent as feeding advanced. The secretion granules of these cells (d, e and f) are presented similar to above said morphology, dimension and coloration in type II acini. Additional cell types are (e cells), which these cells contain the largest secretion granules

observed in the acini and are weakly stained by eosin. Finally are (f cells), which secretion granules in these type of cells, are not seen in feeding stage of female ticks. The cytoplasm of (f cells) was stained uniformly by eosin and the nuclei are round-shaped, presenting dispersed chromatin (Figure 6).

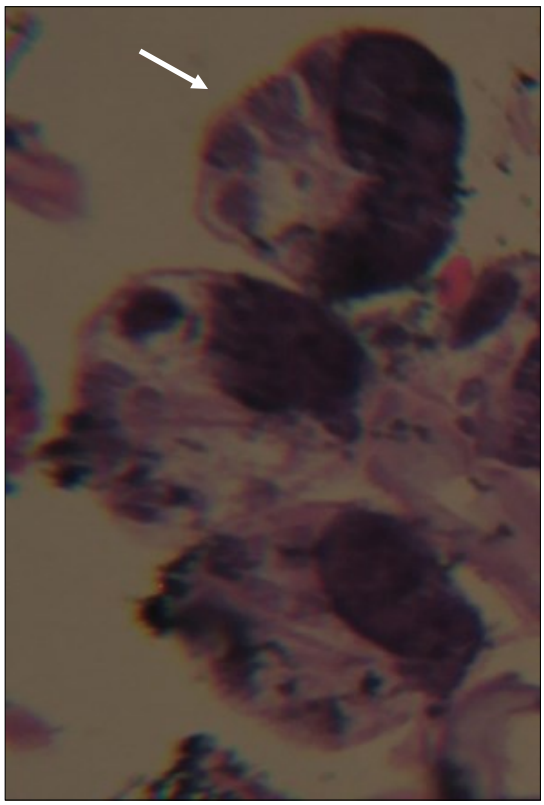

Figure 6. Salivary gland. Hyalomma anatolicum anatolicum female tick. Type III acini. The homogeneous cytoplasm and round-shaped nuclei (arrow) were seen, (H&E×400).

Discussion

Many contradictions have arisen in the grouping of various cell types in the multiple salivary gland acini. Different methods and criteria applied for denominating of the cells. Accordingly, it is very difficult to exactly match existing results with those of other researchers. Nonetheless, an effort has been made to categorize the several cell types in the nominations by (Šimo et al, 2017, Nunes et al, 2006 and Nodari et al, 2012). The concern in the survey of the salivary glands of ixodid ticks has grown, formerly it is there that the combination of molecules with immunological and pharmacological attributes responsible for the compilation of the haemostatic and immune-inflammatory systems of the host happens, and these operations engage the achievement of vitiation. In addition it is important to support the value of tick members in the storage and transfer of pathogens responsible for transmitting infections to several groups of animals, including human (Kazimírová, et al, 2012). At the moment the molecules of the salivary glands have also been checked more to be applied to the cure of diseases, as cancer. The structural specifications of cells in type I acini (Ben Said et al, 2012) protects the assumption of Bowman & Sauer(2004) that the type I acini in unfed ticks are accountable for the making of hygroscopic saliva to absorb water steam from the aerosphere above the climacteric balance moisture. In this study morphologically based on various acinar types the salivary glands of the females H. a. anatolicum were composed of two types of cells, (agranular type I) and (granular types II and III). This is in accordance with the general organization of salivary gland in female Haemaphysalis leachi, which have one agranular and three types of granular acini. It is supposed that the type I acini are accountable for the discharge of hygroscopic saliva in non-parasitic phase stage to absorb water from an unsaturated atmosphere (Bowman & Sauer, 2004). The presence of cells with wide basal membrane in folding and direct apical membranes is generic of an epithelium complex in the discharge of hyper osmotic liquids (Hughes, 2003). Aksan et al, (2009)displayed that Hyalomma anatolicum excavatum ticks, which acinus types II and III were approximately devastated by weighty infections with T. annulata were still capable to absorb water steam, then additional ascribing this action to the type I acini. Vancova et al (2010) found a notable decrease in scale of the type I acini during the feeding of Dermacentor variabilis, A. americanum and Rhipicephalus sanguineus; type I acini was smallest in the ultimate step of feeding when water and ion secretion was highest. Pending nutrition, the type I acini underwent little structural conversions as contrasted to types II, III and IV acini. Appendix sticking the lipid and glycogen-like material existing in the central and circumferential cells vanished subsequently. It is feasible that they operate as power stocks for the ATPase pump during non-parasitic steps, which is discharged during rehydration, or perhaps they probably act as the real hygroscopic substance or its pioneers (Sarah &.Randolph, 2010). Alexandre (2001) viewed mass of lipid inclusions in type I acini of dehydrated A. americanum which absconded on hydration. The lipid inclusions were invisible from type I acini of Boophilus microplus, a one-host tick where fed larvae and nymphs remain on the host to exuviate that is interesting (Kluck et al, 2010). It does not specify the importance of multiple Golgi bodies in relation to congestive vacuoles, myelin and residual bodies in the centric cell, anyway, they perhaps related with autophagy. The results of this survey were emphasized by histological methods can also a simple way to determine of tick salivary glands and a comparative study on it.

Ethics: I hereby declare all ethical standards have been respected in preparation of the submitted article.

Conflict of Interest: The authors declare that they have no conflict of interest.

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