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A histopathological description of *Amblyomma sculptum* attachment site on the skin of a mare at different moments

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ABSTRACT

Ticks' saliva presents immunomodulatory activity and cause several tissue changes at the attachment site on the host skin. Here, we compare different moments of the skin of a Mangalarga Marchador mare before, during and after tick attachment. At the attached site, it was observed inflammatory infiltrate, degranulated mast cells, areas with an absence of nucleus and the presence of pyknotic nuclei, and thick type I collagen bundles around the tick's hypostome, fibrosis, and necrosis. In the skin after the tick detached, we have also observed a complete loss of the structure of the extracellular matrix due to the formation of edema, a large presence of eosinophils and macrophages, new blood vessels, and dilated vessels. These findings indicate that in the absence of immunomodulation promoted by tick saliva, the animal's organism can start the skin repair and wound healing process.

1. Introduction

Ticks are important ectoparasites of horses (Keirans, 1992; Molento, 2005). In Brazil, one of the tick species frequently found in these animals is *Amblyomma sculptum*, known to act as a vector for *Rickettsia rickettsii* (Oliveira and Borges, 2011; Esteves et al., 2019). When ticks penetrate the host's skin with their oral apparatus and release salivary secretion, they cause laceration of tissues and blood vessels resulting in alteration of the skin structure (Moraes et al., 1991). Even with the damage caused to the tissues, the ticks are able to remain feeding due to the properties of the saliva that deflect the immune response of the host due to the anti-inflammatory, immunosuppressive, anti-hemostatic, and vasodilator molecules (Bowman et al., 1997; Chmelar et al., 2012; Kazimírová and Štibrániová, 2013; Nuttall, 2019; Valenzuela, 2004). As a result of tick infestations, macroscopically, defects in the quality of the leather can be observed, such as scar formation, changes in thickness, length, and density of coat (Bayford et al., 1992; Steelman, 1976; Veríssimo et al., 2002; Rehbein et al., 2003). However, little is known about the microscopic aspects of the ticks' attachment site. This study described histological changes between the integral skin of a mare and the skin where ticks are attached/detached at different moments.

2. Materials and Methods

Skin fragments were collected from a 3.5 years-old Mangalarga Marchador mare, weighting 295 kg and pre-sensitized with ticks. The animal was fed on a ration composed of corn and soybean meal, mineral salt, and water ad libitum and was kept on pasture composed of *Cynodon* spp. and *Cynodon dactylon*, approximately 50% of each, in the Teaching, Research, and Extension Unit in Equideoculture of the Federal University of Viçosa, Viçosa, Minas Gerais, Brazil.
At the beginning of the research, the animal was preventively treated with moxidectin plus praziquantel oral gel (Equest Pramox, Zoetis), cypermethrin pour-on (Aciendel Plus, Biogénesis Bagó) and topic deltamethrin (Butox P CE25). The mare was monitored daily for 55 days as follows: 1) monitor the loss of ticks due to treatments, considering the safety interval of approximately 7 to 28 days (Pereira et al., 2008), 2) the new natural infestation occurred – the selection of biopsy sites was based on the monitoring of the engorgement adult females of *A. sculptum*, which was approximately 7 to 10 days after the initial attachment.
Full-thickness excisional skin wounds of 10 mm were performed using biopsy punch in the perianal region after locally anesthetized with 10% lidocaine and disinfection with chlorhexidine. One wound was performed on an intact skin site, one wound was performed on the attachment site of a partially engorged female of *A. sculptum* (six days after attachment), and other wound was performed on the attachment site of an engorged female of the same species, shortly after detachment (eight days after attachment and two days after detachment). The wounds were sutured and treated with antiseptic spray and repellents.

Three samples collected from the wounds were fixed in formaldehyde solution buffered in 0.1M sodium phosphate (pH 7.2) for 24 h, dehydrated in an increasing series of ethyl alcohol, diaphanized in xylol, and immersed in paraffin. Histological sections of 4 µm were obtained on a microtome Leica Multicut 2045 Reichert-Jung (Jena, Germany). One of every 10 sections was used to avoid repeating the analysis of the same histological area. These sections were mounted on histological slides and stained with Hematoxylin and Eosin (Gonçalves et al., 2010), Sirius Red (Dolber and Spach 1993), and Toluidine Blue (Junqueira and Carneiro, 2005). The slides were analyzed in a BX-60 Olympus microscope (Tokyo, Japan).

The tick species attached in the collected skin fragments were identified as *A. sculptum* using a stereoscopic microscope and according to Barros-Battesti et al. (2006). The procedures were approved by the Animal Ethics Committee of the Federal University of Viçosa, license number: 88/2019.

3. Results

Histopathological examination of the intact skin compared to the fragments with attached tick show that the species *A. sculptum* disrupted the skin, besides severe damage to the skin of this animal. This occurs by the penetration of the hypostome and formation of the “cementum cone”. In the attachment site of a partially engorged female tick (six days after attachment), we observe the occurrence of extravasation of red blood cells and inflammatory infiltrate in the dermis (Figure 1). We also observed areas with the absence of nucleus and the presence of pyknotic nuclei that indicates necrosis (Figure 1). While in the attachment site of an engorged female, shortly after detachment (eight days after attachment and two days after detachment), there were new blood vessels and dilated vessels (Figure 1). In addition, the inflammation evolved into edema of the inflammatory exudate characterized by the leakage of plasma and inflammatory cells into the interstitium surrounded by an intense inflammatory infiltrate (Figure 1).

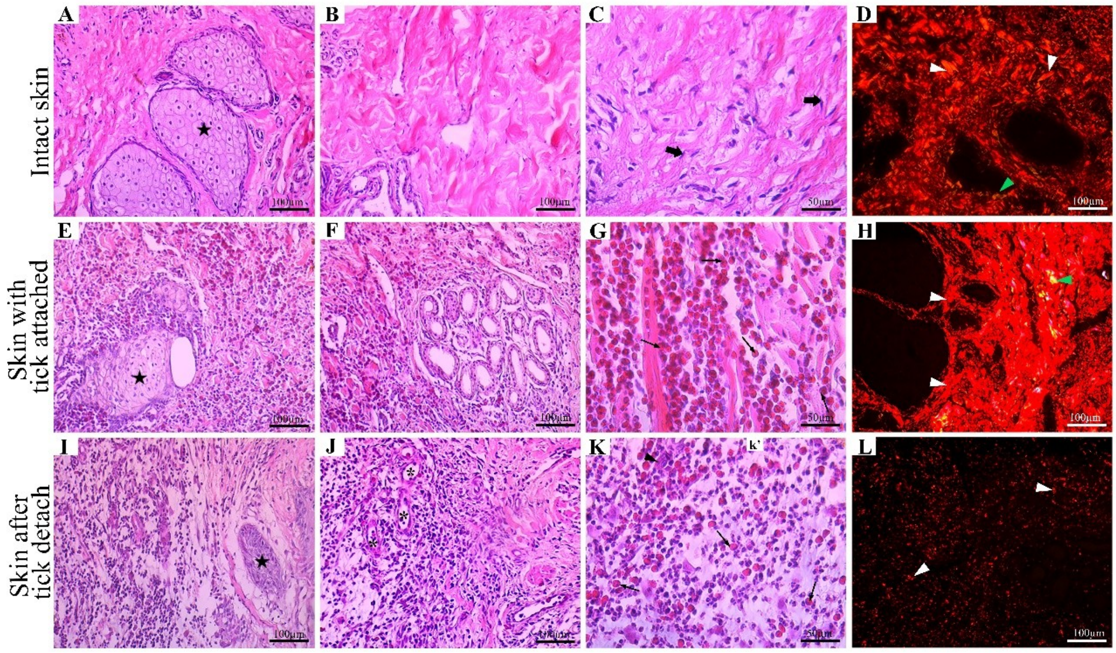

Figure 1 *– Photomicrographs of the skin of Mangalarga Machador in three situations. A, B, C (Hematoxylin and Eosin staining), and D (Sirius Red stain) depict intact skin, composed of characteristic connective tissue with fibroblasts and organized collagen fibers. Figures E, F, G (Hematoxylin and Eosin staining) and H (Sirius Red staining) represent skin photomicrographs during the parasitism of Amblyomma sculptum (six days after fixation). It is possible to observe tissue disorganization where the collagen fibers are without an organizational pattern and the fibroblasts are extremely reduced. Figures I, J, K (Hematoxylin and Eosin staining) and L (Sirius Red stain), show photomicrographs of the skin region after parasitism (eight days after fixation and two days after detachment). The tissue is disorganized, with a drastic reduction of collagen fibers. Star: sebaceous glands; Asterisk: blood vessels; Large arrow: fibroblasts; Thin arrow: eosinophils; Black arrowhead: macrophage; White arrowhead: collagen type 1; Green arrowhead: collagen type 2.*

In the fragment of intact skin, the collagen bundles show homogeneous thickness and distribution. While in the others fragments in which the tick was attached, in addition to the signs of necrosis, we observed changes in the fibrous components of the matrix. In the attachment site of a partially engorged female tick the type I collagen bundles are quite thick around the tick's hypostome. And in the attachment site of an engorged female shortly after detachment occur complete loss of the structure of the extracellular matrix due to the formation of inflammatory exudate (Figure 1). Few mast cells were observed in intact skin, however, in the different moments of tick attachment, we observed the presence of degranulated mast cells (Figure 2).

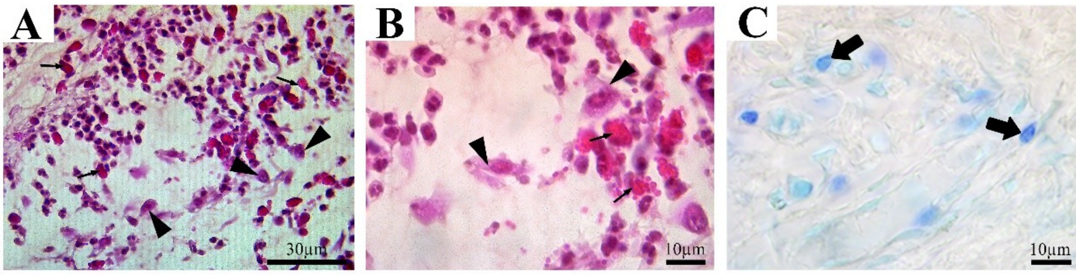

Figure 2 – *Photomicrographs of the skin of a Mangalarga Machador mare (A) six days after attachment, and (B) eight days after attachment and two days after detachment) of Amblyomma sculptum parasitism (Hematoxylin and Eosin stain). The tissue is disorganized with few collagen fibers, and the presence of eosinophils and macrophages. (C) (Toluidine blue stain) It is possible to observe degranulated mast cells. Thin arrow: eosinophils; Arrowhead: macrophage; Large arrow: mast cells.*

4. Discussion

The histopathological changes reported here probably occurred due to the anti-inflammatory, immunosuppressive, anti-hemostatic, and vasodilator molecules present in the tick saliva (Bowman et al., 1997; Chmelar et al., 2012; Kazimírová and Štibrániová, 2013; Nuttall, 2019; Valenzuela, 2004). Studies on the saliva composition of some tick species described the presence of proteins from the lipocalin superfamily. These proteins have the potential to modify the hemostasis, inflammation, and host immunity, through the interference in biogenic amines, such as serotonin and histamine (Chmelar et al., 2012; Mans et al., 2008; Mans and Ribeiro, 2008; Paesen et al., 1999; Sangamnatdej et al., 2002).

In our study, we observed several alterations in the animal's skin due to tick attachment. When the tick penetrates the hypostome into the host's skin, the formation of the “cementum cone” begins, which is composed of a fast-hardening substance produced by the tick salivary glands of the Ixodidae family. These substances probably promote tissue changes in the matrix that stimulate the collagen deposition around the hypostomium, that allows the tick to hang on (Arthur, 1970; Whitwell, 1978). In addition to this process, the cementum helps sealing the wound to prevent fluid loss and increases the effectiveness of blood meal (Kim et al., 2014; Suppan et al., 2018).

In the first days of the tick attachment, there is a process of establishing that precedes the blood meal. During this process, ticks may feed slowly and promote the release of molecules that neutralize the histamine released by the host's mast cells. Thus, the tick prevents the host expelling/rejecting mechanism by inhibiting the vasodilation, vascular permeability, leukocyte recruitment, and edema formation (Chmelar et al., 2012; Mans et al., 2008; Mans and Ribeiro, 2008; Paesen et al., 1999; Sangamnatdej et al., 2002). On the other hand, after the avoidance process, the tick needs to rapidly feed on blood, promoting the release of molecules that stimulate histamine production by the host, which increases vasodilation so that the tick feeds on the blood more easily (Dai et al., 2010). Our histopathological findings suggest that processes similar to the one described for other tick species can also occur with *A. sculptum*. Since the attachment site of a partially engorged female tick presents different characteristics from those observed in the site of an engorged female detached. In addition, there is a possibility that in the fast-feeding phase, the formation of edema can be prevented by other tick salivary molecules (Dai et al., 2010). However, our finding shows that edema had already occurred, which probably contributed to the expulsion of the engorged female.

The changes in the extracellular matrix in the attachment site of a partially engorged female tick indicate a fibrotic process and the occurrence of cell death by necrosis (Aziz et al., 2016; Hargis and Myers, 2017; D’Arcy, 2019; Nogueira et al., 2021), which is a process mentioned at the location of other tick species (Walker and Fletcher, 1986; Latif et al., 1991). The extravasation of red blood cells in the dermis, observed where the partially engorged female was feeding, probably occurred due to the salivary secretion of the ticks being a source of anti-hemostatic molecules so that it is possible to feed since the host releases several hemostatic molecules (Chmelar et al., 2012). The mast cells, present both in this fragment and in the skin fragment in which the tick has already detached, have granules that store various molecules, including the histamine that is released when degranulation occurs (Borriello et al., 2017), which corroborates with the previously mentioned. In addition, these cells act by cutting inflammatory cells to the lesion site (Shiota et al., 2010; 2005) and play an important role in the response to pathogens, ticks, and other ectoparasites (Borriello et al., 2017).

As previously mentioned, the edema seen here, must have started in the fast phase of feeding the tick and intensified after its expulsion. This is probably due to the absence of immunomodulation promoted by tick saliva, the animal's organism can start the repairing and healing process. This is a complex process but extremely important because for the repair process to occur it is necessary the appropriate degradation of the extracellular matrix. So that the new matrix can be synthesized. This process is divided into the following phases that can overlapped: hemostasis, inflammation, proliferation, and remodeling (Oryan et al., 2016). The process observed after the detachment of the partially engorged female tick was found in the initial phases of hemostasis and inflammation, responsible for the cleaning of wound remains by the inflammatory cells. We saw an intense presence of eosinophils, which are important modulators of hypersensitivity reactions (Chusid, 2018), and macrophages. These would act in phagocytosis and play an important role in the angiogenesis that is already occurring, as observed by the presence of new and small vessels.

In the subsequent processes due to chemokine secretion, such as matrix deposition, fibroblast migration and re-epithelialization can be also seen (Wynn and Vannella, 2016). Although our histological findings do not show total tissue recovery, in the next phases other important processes can take place, such as in the proliferation phase, fibroblasts would migrate, and granulation tissue and provisional matrix would be formed. All these could be replaced in the remodeling phase by the new extracellular matrix, characterized by scar tissue rich in collagen (Kawasumi et al., 2012).

5. Conclusion

The attachment of the tick in the skin of the animal caused marked histopathological changes. We were able to visualize the immunomodulatory action of tick saliva, because in the absence of immunomodulation, the animal's organism can start the skin repair and wound healing process. The present study has improved our knowledge about the host-parasite relationship.

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