

A COMPARATIVE STUDY OF LESIONS CAUSED BY DIFFERENT PARASITIC STAGES OF *Boophilus microplus* (CANESTRINI) IN THE SKINS OF NATURALLY INFESTED TAURINE AND ZEBUINE HOSTS. THE CORRELATION OF TICK RESISTENCE WITH MAST CELL COUNTS IN THE HOST'S SKIN

ESTUDO COMPARATIVO DE LESÕES CAUSADAS PELOS DIFERENTES INSTARES DE *Boophilus microplus* (CANESTRINI) NA PELE DE TAURINOS E ZEBUÍNOS EM INFESTAÇÕES NATURAIS. CORRELAÇÃO ENTRE A RESISTÊNCIA DO HOSPEDEIRO E O NÚMERO DE MASTÓCITOS DÉRMICOS

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SUMMARY

Biopsies of skin injuries caused by larvae, nymphs, unengorged and engorged females of *Boophilus microplus* were collected from five *Bos taurus* and five *Bos indicus* steers, which had been born on the same ranch, aged about 23 months and had been grazing together in the same pasture during the preceding 90 days. Their susceptibilities to the tick had been previously estimated, being the taurine animals around fifteen times as susceptible as the zebuine ones. The biopsies were made in such a manner that the mouth pieces were in the center of the skin fragments. These were immersed for 24 hours in 10% formalin, then processed through usual histopathological techniques and stained either by the haematoxylin-eosin or by Masson's "Trichromic" procedures. No morphological differences were found capable of explaining the higher tick susceptibility observed for the taurine hosts as compared to the zebuine's. Biopsies from non parasitized skin from the groin region of five *B. taurus* and five *B. indicus* steers were fixed in 1% lead subacetate, plus 50% ethanol, plus 1% acetic acid, for 24 hours, which they were histologically processed and stained in 0.1% toluidin blue. The mast cells were comparatively counted in 80 microscope fields per sample with 100 diameters of magnification and the counts averaged more than twice as high in the zebuine than in the taurine host's skins. These results strongly suggest that mast cells participate as important elements in the bovine host's resistance to the cattle tick.

UNITERMS: Skin diseases; *Boophilus*; Mast cells; Cattle; Zebu

INTRODUCTION

Bovine hosts present different levels of resistance against infestation by *Boophilus microplus*, the common cattle tick^{21, 24, 32, 36}; such resistance is mostly directed against the larval stage^{25, 26, 27}; at its first 24 hours of parasitism^{5, 16, 25, 26, 33, 34}; and the potentiality for resistance is of genetic nature^{27, 32, 35}.

Upon reaching the host's skin the larvae walk around for one hour or more and then try to attach themselves by means of their mouth pieces, thus beginning their parasitic stage²⁴. According to CASTRO and PEREIRA⁹ (1946), an erosion occurs inside the epidermic layer of the rabbits skin followed by necrosis of the granulous and Malpighian layers, as a consequence of traumatism inflicted by the tick's mouth pieces combined with an energetic plasma exudation attributed to the toxic effect of its salivary secretions; there follows congestion of local blood vessels and the affluence of leucocytes, beginning with the neutrophils, which are later followed by mononuclear cells concomitant with necrosis of the dermis. Since the skin contains histamin in concentrations sufficient to start the first inflammatory manifestations at the tick's attachment site, it follows that the mechanical injury caused by larvae is the initial fact for the triggering of the inflammatory process. Ulteriorly the successive emissions of saliva cause new waves of irritation inside the dermis or in its proximity,

thus provoking, maintaining or aggravating the phlogistic phenomena, which tend to be acute at first and to evolve later gradually to chronicity³⁰. TATCHELL and MOORHOUSE³² (1968) observed in *B. taurus* and in *B. indicus* the skin alterations related to the attachment and feeding of *B. microplus* both in primary infestations and in reinfestations. The lesions on the skin of taurine hosts exposed to attachment of tick larvae showed dilation of capillary vessels with infiltration and rupture of eosinophils, this last aspect being intensified at the reinfestations.

This infiltration was the main component to contrast tick lesions between taurine and zebuine hosts, being significantly more intensive in the taurines for injuries done by the larvae, but not so for the lesions due to the ixodid's nymphal and adult stages. Such eosinophilic reaction was stronger for taurine than for zebuine crossbreds and was interpreted as due to immediate hypersensitivity. This phenomenon occurred particularly with the less resistant individual hosts and, according to TATCHELL and MOORHOUSE³² (1968), it result in a higher supply of fluids and of tissue debris as feed for the larvae.

SCHLEGER et al.³¹ (1976), making observations of similar nature, verified that both in highly resistant animals and in more susceptible ones the number of eosinophils in the inflammatory

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infiltration is similar, but their concentration on the sites close to the tick injury were smaller in the most resistant hosts, and so was the number of broken mast cells. While the destruction of such cells was significantly smaller in hosts of low resistance, the mast cells from an extensive area showed some degranulation, what suggests the diffusion of the salivary antigen. In hosts naive to the tick's parasitism there was a comparative paucity of the number of infiltrated eosinophils, what according to SCHLEGER et al.³¹ (1976) emphasizes the immunitary basis of the reaction, thus corroborating TATCHELL and MOORHOUSE³² (1968). Eosinophils predominate in the injury caused by the larvae, at the initial three hours, there occurring an intensive neutrophil infiltration at the time coinciding with the end of the larval engorgement^{29, 30}.

Mast cells derive from the bone marrow and are residents in all tissues of the organism. They participate of several phenomena such as the reactions of hypersensitivity and other organic reactions against parasitic infections and infestations, particularly against gastrointestinal nematodes, where mast cells of the mucosae play a relevant role^{14, 15, 17, 20, 22, 23, 37}. Mast cells from the connective tissue, dwelling in the dermis, seem also to be involved in the phenomena of resistance to ectoparasites, since SCHLEGER et al.³¹ (1976) observed that susceptible bovine hosts had a smaller rate of degranulation of these cells as compared to resistant hosts when both were exposed to *B. microplus*. Furthermore, the dermic mast cells are implicated in the defense mechanism of the mouse's skin against *Shistosoma mansoni*¹¹.

On the other hand the basophils (which are bone marrow originated cells of the circulating blood) migrate to the tissues where immunitary reactions of immediate hypersensitivity are in course^{9, 10} and to their action together with those of eosinophils and Ig G is attributed the rejection of tick's^{1, 3, 7, 18}. The stimulation exerted by the ixodid causes such cells to be recruited from the bone marrow, through the blood stream, to participate on the local immunitary reaction^{2, 7}. The basophils predominate on the inflammatory focus produced by *Amblyomma americanum* in the skin of guinea pigs four days after attachment. On reinfestations, 24 hours after the tick's attachment such cells approach 60 to 70 per cent of the cellular inflammatory exudate⁶ and degranulation of basophils was seen on the sixth hour after tick attachment, being 90 percent to these cells degranulated within 72 hours⁷.

The objective of this study was to examine the histopathologic aspects of injuries produced by the different parasitic stages on *B. microplus* in the skin of taurine and zebuine hosts naturally exposed to the infestation by this species of tick, against which the levels of susceptibility of those hosts had been previously estimated. The mast cells in the dermis of each host were quantified by means of skin biopsies uniformly collected from the integument of the groin region, with due care to get samples free from tick infestation and from any noticeable sign or sequel of a previous local tick infestation. Mast cell comparative count was employed as a new criterion to contrast tick resistant (zebuine) and tick susceptible (taurine) hosts.

MATERIAL AND METHOD

Ten steers aging about 23 months, five of them being zebuine of Gir breed and the other five taurine of Holstein breed, all born in a same ranch, had been previously compared as to tick natural resistance against *B. microplus* infestation, with

the result that the first averaged about 15 times as resistant as the last²¹. Before the start of the present study the ten steers were sprayed with an anti-tick preparation (Amitraz at 12.5%). Dully identified by numbered ear-tago they were grazed together in a fenced pasture known to harbour *B. microplus* larvae, where they were kept up to the end of the experiment, receiving no other antiparasitic treatment.

Around the 90th day after grazing in that pasture the taurine and zebuine animals were brought to the corral and from each of them were taken two skin biopsies with attached larvae, two with nymphs, two with tick females shorter than 4.0 mm (non-engorged) and two tick females large than 4.0 mm (engorged), in such a manner that at least one tick in each biopsy was attached to the center of the skin fragment. The fragments were immersed in 10% formalin for 24 hours and then processed according to the usual histological techniques for the preparation of serial sections of 6.0 µm of thickness from paraffin blocks, followed by staining by the Haematoxylin-Eosin or Masson's "trichromic" methods.

For mast cell quantification in the dermis, skin biopsies were taken at the groin region both of the taurine and zebuine hosts, fixed in 1% subacetate, plus 50% ethanol, plus 1% acetic acid. After 24 hours fixation they were processed by the conventional histologic techniques and paraffin-block sections with 6.0 µm thickness were serially prepared with a minimum interval of 20 µm between consecutive sections. From each series four sections were prepared and stained by 0.1% toluidin blue. The mast cells were counted in four different microscope fields, taken at random, from each of the four sections from every sample, totalizing 80 counts per sample at the augmentation of 100 diameters. The comparative counts for zebuine (tick resistant) and taurine (tick susceptible) skin sections were statistically analyzed by the simple linear regression test, with calculation of the variation coefficients.

RESULTS

THE MICROSCOPY OF INJURIES CAUSED BY LARVAE ON THE SKINS OF TAURINE AND ZEBUINE HOSTS

For both cattle sub-species a cement cone was implanted at the interface between the corneous and granular skin layers, enveloping the tick's mouth pieces and allowing to recognize the hypostomium and some of its denticles. Immediately below there was total destruction of the epidermis and part of the papillary layer, followed by a zone which consisted of necrotic material and cellular debris. Around this process there was an inflammatory infiltration consisting mostly of mononuclear cells, having among them a small number of polymorphonuclear neutrophils; hyperemia was noted with leucocytary margination of the blood vessels and perivascular infiltration of mononuclear cells. The qualitative composition of the exudate was basically the same in almost all the observed samples with the exceptions of two zebuine skin biopsies in which eosinophils were detected, a type of cell not observed in tick lesions from taurine hosts.

THE MICROSCOPY OF INJURIES PRODUCED BY NYMPHS IN THE SKIN OF TAURINE AND ZEBUINE HOSTS

As in the above situation, the cement cone implanted itself in the intermediate position of the corneous and granular layers, but it was of larger dimensions than that secreted by the tick larva. The nymphal mouth pieces were also wrapped by the

cement cone in this one being impressed the marks of the hypostomium's denticles. The epidermic layer was entirely destroyed and below there was a cavity containing cell debris; the collagen fibers both from the papillar and reticular layers were disrupted, there existing also a cellular inflammatory infiltration with polymorphonuclear neutrophils as the predominant component. In two taurine biopsies and in one from a zebuine host the inflammatory infiltration presented a discrete tissue distribution and a moderate number of cells, mainly mononuclear cells, involving also the hair, follicles, sebaceous glands and blood vessels, being last dilated, congested and containing marginated masses of white cells.

THE MICROSCOPY OF LESIONS INFLICTED BY NON-ENGORGED FEMALE TICKS SHORTER THAN 4.0 MM, IN THE SKIN OF TAURINE AND ZEBUINE STEERS

These lesions were salient to the epithelial surface; the cement cone implanted itself on the interface of the corneous and granular layers, protruding into the last and proportionally larger than the ones produced by larvae and nymphs. The destruction of the epidermic layer was complete at the tick's attachment site. There was intense congestion of blood vessels which contained large number of leucocytes in marginal disposition; a dense inflammatory exudate was present and composed mainly of mononuclear cells but having among them a smaller number of polymorphonuclear neutrophils. The rupture of collagen fibers was also observed in the papillar and reticular layers. The cellular exudate involving the sebaceous glands, the ducts of the sudoriparous glands and the hair follicles was neatly limited to the deeper zones of the reticular layer which presented itself moderately infiltrated by neutrophils.

THE MICROSCOPY OF LESIONS CAUSED BY ENGORGED FEMALE TICKS LONGER THAN 4.0 MM, IN THE INTEGUMENT OF TAURINE AND ZEBUINE HOSTS

An increase in volume was observed at the bite's location which became salient to the epithelial surface. As in the above cases, a cement cone was present, but rather larger involving the mouth pieces of the tick and entering deeply into the reticular layer. There was complete destruction of the epidermis followed by a necrotic ulcer with intense fragmentation of the collagenous fibers, ample accumulation of cellular detritus, and phenomena connected with congestion and haemorrhage, either discrete or diffuse. Circumscribing such aspects or penetrating in them there was a dense inflammatory cellular infiltrate mainly composed of mononuclear cells and containing among them a large number of polymorphonuclear neutrophils. All of these alterations were neatly limited to zones inferior in deepness to the one marked by the presence of sudoriparous glands. In two cases spherical cellular remains markedly basophilic were seen in the "lumen" of the cement cone; in two other cases was observed predominance of polymorphonuclear neutrophils in the cellular inflammatory infiltrate. In two of the skin biopsies from zebuine hosts were observed eosinophils within the deeper regions of the reticular layer, specially around the blood-vessels.

NUMBER OF DERMIC MAST CELLS IN TAURINE AND ZEBUINE HOSTS

The results of mast cells average counts in the dermis at the groin region of five taurine and five zebuine steers are condensed in Tab.1, and clearly shows that in the last the number of such cells is above twice as many as that counted in the first.

TABLE 1

Numbers of dermic mast cells in the skin of the groin region of five taurine and five zebuine steers, as expressed in average counts (x) per sample and standard errors of the averages from eighty counts for each sample.

Sample n°	Taurine	Zebuine
1	47.9±2.59	91.3±5.23
2	69.0±2.93	143.5±2.55
3	23.1±1.91	112.4±6.93
4	41.4±2.45	110.1±11.3
5	36.1±1.51	93.9±6.08
X	43.1±2.28	110.2±7.1
Total	217.5	551.2

DISCUSSION

Through the microscopic observation of lesions inflicted in the skin either of taurine or zebuine steers by the tick *B. microplus* at each of the parasitic stages of its life cycle, none or very scarce significant morphologic differences were observed which could perhaps be connected with the host's subspecies, though to its *B. taurus* or *B. indicus* nature. Only in two of the five samples of injuries caused by tick larvae in the skin of the zebuine hosts were seen eosinophil cells, which were not, on the other hand, found in the comparative preparations of skin injuries inflicted by larvae in taurine steers. Similar findings occurred in skin samples infested by female ticks when these were not fully engorged (tick females shorter than 4.0 mm), but in these last cases the neutrophils appeared exclusively in the deeper zones of the reticular layer, mainly around the blood vessels; such findings are not though, enough to establish relationships between the host's levels of susceptibility to the tick and the inflammatory alterations this tick causes in those host's skins.

TATCHELL and MOORHOUSE³² (1968) employing experimental *B. microplus* infestations and reinfestations comparatively in taurine and cross-bred zebuine x taurine hosts, verified that infiltration and rupture of eosinophils, intense for purebred taurine animals at the reinfestations, was the only aspect in the inflammatory process caused by the tick larvae to permit a differentiation in the reaction between the two host types. They interpreted this intense taurine's reaction as originated by a process of immediate hypersensitivity. Such process would result in a higher tissular fluid disposibility as food for the larvae, thus enhancing their development.

SCHLEGER et al.³¹ (1976), on the other hand, in a similar study, arrived at about the same eosinophil counts in tick lesions both of susceptible and resistant host's skins but observed that in the immediate neighbourhood of the attachment site of the larva the concentration of those cells was significantly higher for the more resistant host what would result in severe pruritus for such a host.

There is contradiction between results from these different authors, what calls for new observations, particularly covering Brazilian zebuine breeds and mostly on what concerns the morphology of skin injuries by the tick's larva, the stage against which seems to be directed most of the host's defence mechanisms.

The results of the present experiment indicate that the feeding of the three parasitic stages of the tick occurs in three phases: 1st since the larva is histophagic there is a local tissue digestion, particularly of the epidermis and reaching the pap-

illary layer, what is performed either by means of pharmacologically active components of the tick's saliva or by the action of chemical mediators locally liberated, activated or there newly formed; 2^d after the inflammatory leucocytic infiltration, the ingestion by the tick of the host's tissue fluids a great number of degraded leucocytes are transformed in basophilic spherical bodies which are found close or within the cement cone's "lumen" or inside the tick's caeca; 3rd and, as the nymphs and adults, mostly the females, ingest large volumes of blood, this one and the above described elements compound together the whole picture.

Therefore the structural alterations on and around the tick's attachment site are the result not only of the traumatic injury by itself or pharmacologically active substances injected by the parasite, but are also the consequence of the host's inflammatory reaction. TACHELL and MOORHOUSE³³ (1970) showed that dogs made leucopenic by the treatment with nitrogenated mustard suffered from marked inhibition of the inflammatory reaction to *Rhipicephalus sanguineus* infestation when compared with dogs not so treated. When in treated ones the neutrophils counts returned to the normal values the inflammatory reaction of the host to the parasite's bite also reverted to its primitive characters. Therefore, TACHELL and MOORHOUSE³³ (1970) concluded that the neutrophils have a fundamental participation on the structural alterations inflicted by the ixodid in the dog. BECHARA e SZABÓ⁴ (1985) observed, besides, in inflammatory reactions determined by the saliva of engorged *B. microplus* females in the rat, that histamin and some metabolites of the arachdonic acid play a relevant part.

The results presented here of dermic mast cell counts Tab. 1 show that in zebuine hosts they amount to more than twice the number found in taurine hosts per unit of area in the respective healthy skins of the groin region.

It is a known fact that mast cells and basophils are implicated in the pathogenesis of immediate hypersensitivity reactions^{9, 10}. When these cells are sensitized by Ig E and in some cases also other classes of antibodies developed against specific antigens, they quickly liberate histamin and serotonin, as well as other substances supposed to act as powerful mediators of the inflammatory process^{12, 13, 19}. These cells are also involved in several other morbid processes in which the liberation of mediators is induced needlessly of the activity of any specific antigen, as, for instance, by ceratin fragments of the complement chain, by the poisons of some insects and by other basic peptid¹².

Several writers attribute the rejection of ticks by their hosts to the combined action of basophils, eosinophils, T lymphocytes and also Ig G^{1, 3, 7, 18} since such cells and particularly the basophils predominate in the inflammatory focus which follows the reaction to the saliva from several tick species. The basophils are not tissue resident and need to be recruited from the bone-marrow through the blood stream, to come and participate at the local immunitary reaction^{2, 7}. They predominate in the inflammatory infiltrate caused in the skin of guinea-pigs, on the 4th day of infestation by *Amblyomma americanum*, when about 80% of the attached ticks are rejected. At the reinfestations the basophils are already the predominant cells 60 to 70 per cent of the infiltrate after 24 hours of the tick attachment. Eosinophils are at this time the second type of cells (around 10 to 20, per cent of the infiltrate)⁶. In an electronmicroscopic study BROWN and KNAPP⁶ (1981) observed that 6 hours after tick affixation some basophils presented themselves already degranulated.

In the above papers, though, no mention is made to dermic mast cells as entities capable of interfering on the host's mechanisms of resistance against the parasitism by ticks, but it must be remembered that GERKEN et al.¹³ (1984) found that mice deprived from their dermic mast cells became more susceptible to *Schistosoma mansoni* infection, through skin penetration by the trematode's cercariae. SCHLEGER et al.²¹ (1976) refer to the mast cells in the infiltrate surrounding the larvae's points of attachment. The number of such cells is larger in resistant than in susceptible bovine hosts, an observation not confirmed on the present experiment. CASTRO and PEREIRA⁹ (1946) referred, besides, from experimental infestations in rabbits, that the skin contains enough histamin to trigger the first inflammatory reactions at the site of affixation of the *B. microplus* larvae, and BECHARA and SZABÓ⁴ (1985) showed that such phlogistic mediator, in association with some derivatives of arachdonic acid, play a relevant part in the inflammation caused in rats by the saliva of *B. microplus* engorged females.

As was already pointed out the basophils are recruited from the bone marrow, via blood stream, to actuate locally at the point of the tick's attachment to the host's skin. These cells mediating a time interval of about 6 hours, the minimum period elapsed before such cells were detected in the inflammatory infiltrate.

On the other hand mast cells reside at the dermis (as in most of the tissues) and their degranulation can be triggered by the combined mechanical trauma caused by the tick's mouth pieces and its injected saliva. As became evident by this study, in zebuine hosts the undamaged skin may harbour above twice as many mast cells per surface unit than in the taurine's uninjured integument. The degranulation of these cells liberate several pharmacologically active substances among them histamin, which besides other effects, provoke intense pruritus. This it teases the animals, into self-licking which is the main mechanism by which they get rid of most larvae and of the other parasitic stages of the ixodid. ROCHA³⁰ (1976) commented that when cattle are - by means of halters and short ropes - restrained from self-cleaning and then experimentally infested with *B. microplus* larvae - the difference between resistant and susceptible hosts becomes less evident and the intensity of the parasitism increases many fold in both resistant and susceptible animals. The same author revealed that the self-cleaning activity through self-licking starts from half an hour to two hours after experimental infestation of unrestrained, resistant bovine hosts. Therefore, this reaction begins long before the basophils can come through the blood stream from the bone-marrow after the tick's stimulus elicited by the parasite's initial bites.

Therefore the evidence found of a much higher number of mast cells in the uninjured skin of zebuine as compared to the number of these cells in the uninjured skin of taurine animals may explain - at least in part - the higher efficacy with which the first get rid of their ticks when compared to the last - and specially so in what concerns the infestation by the larval stage of the ixodid.

RESUMO

Biópsias de pele lesada por larvas, ninfas e fêmeas engurgitadas de *Boophilus microplus* foram colhidas de cinco *Bos taurus* da raça Holandesa e de cinco *Bos indicus* da raça Gir, adqui-

ridos na mesma propriedade, com aproximadamente 23 meses de idade, e colocados em convivência no mesmo pasto durante 90 dias que precederam este ensaio. O grau de susceptibilidade ao ácaro foi estimado previamente, sendo os zebuínos cerca de 15 vezes mais resistentes que os taurinos. As biópsias foram colhidas de modo que o parasito ficasse localizado no centro do fragmento de pele. Essas peças foram fixadas em formalina a 10% durante 24 h e processadas pelos métodos usuais em histologia seguindo-se a coloração pela técnica da Hematoxilina-Eosina ou do "tricroômico" de Masson. Não foram constatadas diferenças morfológicas que pudessem ser relacionadas aos diferentes graus de resistência ao parasito entre os taurinos e zebuínos. Biópsias foram colhidas de zonas não lesadas, na região da virilha dos cinco taurinos e dos cinco zebuínos, fixadas em subacetato de chumbo a 1% em solução de etanol 50%, contendo 1% de ácido acético, por 24 h. A seguir os fragmentos foram processados pelas técnicas usuais em histologia e corados pelo método do azul de toluidina 0,1%. Os mastócitos foram contados comparativamente em 80 campos microscópicos, em aumento de 100 vezes. Os resultados mostram que os zebuínos tem mais que o dobro do número de mastócitos dérmicos quando comparado com o encontrado nos taurinos. Esses resultados sugerem que os mastócitos dérmicos possam, pelo menos em parte, participar dos mecanismos de resistência dos bovinos ao *Boophilus microplus*.

UNITERMOS: Pele, doenças; *Boophilus*; Mastócitos; Bovinos; Zebu

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