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Mast cell counts correlate with *Rhipicephalus (Boophilus) microplus* tick load in different cattle breeds

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Abstract

The number of dermal mast cells in several European and Zebu cattle breeds was determined and correlated with the tick load. Breeds included European registered and non-registered Holstein, Brown-Swiss and Jersey cows, Zebu (Nelore and Gyr) and crossbred cows with either high or low tick infestations. Animals were naturally infested with *Rhipicephalus (Boophilus) microplus* and evaluated at regular time intervals for tick attachment and feeding. Ear biopsies were taken and processed according to routine histology for mast cell countings carried out on both the upper and deep dermis. The average cell counts were then correlated with the number of engorged ticks attached to host. Nelore cows presented the largest mast cells countings/mm2 (139.42; $P < 0.05$), while surprisingly Gyr showed similar number of cells (61.73) as the Holstein (48.76) and Brown-Swiss (67.31) ones. Jersey cows presented the smallest cell countings (29.32) despite their greater resistance to tick infestation as compared to Holstein and Brown-Swiss. There was a negative correlation ($r = -0.21$, $P < 0.01$) between the number of cells in the upper dermis and tick counts when all data (n= 155) were analyzed in a single correlation plane, irrespective of the breed. These results confirm the important role played by mast cells in bovine resistance to the cattle tick *R. microplus*.

Keywords: Mast cells, *Rhipicephalus (Boophilus) microplus*, zebuine, taurine, crossbred

Introduction

The cattle tick *Rhipicephalus (Boophilus) microplus* causes serious damage to the health of susceptible cattle, mainly breeds (Bos taurus) of European origin and their crossbreeds. On the other hand, Zebu (Bos indicus) breeds are naturally resistant to this parasite (25).

Mast cells, and the histamine they contain inside cytoplasmic granules, are of fundamental importance to the self-grooming mechanism, which in turn is critical to the resistance of the animals to the *R. microplus* tick (24).

The number of mast cells in the skin varies according to the breed. Moraes et al. (12), analyzing skin biopsies from the groin area, and Sartor et al. (17) who investigated tissue samples taken from the inside of the ear, showed that Gyr cattle had more than twice the number of dermal mast cells than Holstein cattle. Lavielle et al. (9) investigated skin biopsies from the costal region and found that Indubrasil cattle (Zebu breed) had more than twice as many superficial dermal mast cells than F1 crossbreds (Holstein x Indubrasil). Engracia Filho et al. (3) also found a significant negative correlation between the size of the upper dermal mast cell population and the number of ticks on the skin of F2 Holstein x Gyr crossbred cattle after artificial infestation with 10,000 *R. microplus* larvae per animal.

The study of characteristics potentially associated to the resistance of cattle to the *R. microplus* tick is crucial and may be further developed into an important tool for breed selection.

The objective of this work was to determine the number of mast cells in the skin of some European (Holstein, Brown-Swiss, Jersey), Zebu (Nelore, Gyr) and crossbred (European x Zebu) cattle breeds and correlate them with *R. microplus* tick infestation.

Material and methods

Animals

The study was based on the data collected from 117 Nelore cows, 63 Gyr dairy cows, 39 registered Holstein (R) and 24 non-registered Holstein (NR), 37 Brown-Swiss, 166 Jersey and 12 crossbred cows. Animals were adults with age varying between 3 and 8 years old. From this total, 29 Nelore (25.6%), 25 Gyr (39.7%), 21 Holstein (R) (53.8%), 20 Holstein (NR) (83.3%), 21 Brown-Swiss (56.7%), 28 Jersey (16.9%) and 11 crossbred cows (91.6%) were selected for biopsy as they presented the highest and lowest tick infestation average values in each herd.

With the exception of the Jersey herd, the cows belonged to the Instituto de Zootecnia (IZ), located in Nova Odessa, São Paulo State, Brazil (22º42'S and 47º18'W).

The Holstein (R) and Brown-Swiss cows - registered by the respective Brazilian Breeders Associations - were raised together in an intensive milk production system and kept, most of the time, in paddocks and maintained on stall feeding. These cows were derived from cattle imported from the United States in 1982.

The Holstein cows (NR) were not registered and raised together with the Gyr and crossbred cows in a different milk production system, with pasture being the main source of bulk feed. The Nelore cows shared the same pasture of Colonião grass (*Panicum maximum*) as the dry cows of this system.

The Jersey cows belonged to a private herd from the neighboring city of Americana, SP, at a distance of 18 km from the IZ. The Jersey herd was raised in a semi-intensive milk production system and with greater access to pastures of Tanzânia grass (*Panicum maximum* cv. Tanzânia).

Tick Infestation

Natural *R. microplus* tick infestations were evaluated using a simplified count method consisting of counting only the female ticks greater than 4 mm in the fore third of the animal (head, neck, dewlap, shoulder, scapula and arm). These counts are then multiplied the number by three (26). Each cow was evaluated at least twice on the following dates, according to the herd: Nelore (23/08/2000, 20/11/2000); Holstein (R) and Brown-Swiss (10/01/2000, 24/03/2000, 04/12/2000, 15/05/2001, 21/06/2001); Holstein (NR), Gyr and crossbred (04/10/1999, 18/05/2000, 04/10/2000, 24/11/2000); Jersey (06/12/1999, 09/06/2000, 19/10/2000, 06/11/2000).

Skin biopsies and histological processing

Skin biopsies were taken from the inside of the right ear, on days 06/11/2000 (Jersey), 20/11/2000 (Nelore), 24/11/2000 (Gyr, crossbred, Holstein NR), and 04/12/2000 (Holstein R and Brown-Swiss) using a 6mm diameter punch and prior application of local anesthetics (lidocaine hydrochloride 5%). It should be stressed that most of the biopsied skin did not contain any attached tick. The obtained skin fragments were immediately fixed in buffered formaline (pH 7.0) and processed according to routine histologic techniques. Each biopsy was serially sectioned lengthwise at 3 μm intervals and stained with hematoxiline-eosine and May-Grünwald & Giemsa for general morphology and mast cell counts, respectively. The count area of 0.0052 mm2 was delimited with a Reichert eyepiece (Austria/PK 6.3x mm) and a 100x objective. Counts were carried out on both the upper and deep dermis of five areas (5 x 0.0052 mm2).

Statistical analysis

Totally random delineation was carried out, with a different number of repetitions for each breed, for the statistical analysis of the number of mast cells in the dermis (average counts of five areas). For each animal, tick count averages were used to establish a correlation with the number of mast cells in the superficial and deep dermis and total number of mast cells.

Results

Table 1 shows the number of mast cells in the upper, deep and total dermis, according to the group into which each breed was placed: low or high infestation.

Upper dermis mast cell counts

The average numbers of mast cells in the upper dermis per cattle breed are presented in Table 2. The largest number of mast cells was found in Nelore cows ($P<0.05$), followed by Holstein (R), Brown-Swiss, Gyr, Holstein (NR) and crossbred cows. Jersey presented the smallest ($P<0.05$) numbers of mast cells, however, these did not statistically differ from those presented by the Brown-Swiss Gyr, Holstein (NR) and crossbred cattle.

Deep dermis mast cell counts

The average numbers of mast cells in the deep dermis of the breeds investigated are also depicted in Table 2. The Nelore breed also had the largest number of mast cells, however it did not differ significantly from the number found in the Holstein (R), Brown-Swiss and

crossbreds. Gyr, Jersey and Holstein (NR) presented a smaller number of mast cells in the deep dermis, however they did not differ from the number found in the crossbred, Brown-Swiss and Holstein (R).

Total number of dermal mast cells

The average numbers of total mast cells are shown in Table 2 as well. Of all the breeds studied, Nelore exhibited the largest ($P<0.05$) numbers in the dermis, followed by Holstein (R), Brown-Swiss, Gyr, Holstein (NR) and crossbreds, which did not differ from each other. The Jersey cows exhibited the smallest number of mast cells in the dermis ($P<0.05$) and did not significantly differ from the crossbreds, Holstein (NR) and Gyr.

Correlation between number of mast cells and tick infestation

The correlation between the number of mast cells in the upper, deep and total dermis and the number of ticks is presented in Table 3.

Negative correlations were observed between mast cells in the upper dermis and tick infestation in the Nelore ($r = -0.1464$), Holstein (R) ($r = -0.3367$), Brown-Swiss ($r = -0.3231$), Holstein (NR) ($r = -0.0199$), and crossbred ($r = -0.1354$) cows. Positive correlations between these two parameters were only found for Jersey ($r = 0.0867$) and Gyr ($r = 0.1882$) cows.

Correlation between mast cells in the deep dermis and tick infestation was negative for the Nelore ($r = -0.2589$), Holstein (R) ($r = -0.0437$), Brown-Swiss ($r = -0.3084$) and crossbred ($r = -0.0369$) cows. Positive correlations were observed for the Gyr ($r = 0.4224$, $P<0.05$), Jersey ($r = 0.3862$, $P <0.05$) and Holstein (NR) ($r = 0.1403$) breeds.

As for correlation between the total number of dermal mast cells in the dermis and tick infestation, correlation was negative for the Nelore ($r = -0.2275$), Holstein (R) ($r = -0.1944$), Brown-Swiss ($r = -0.3800$) and crossbred ($r = -0.0785$) breeds. A positive correlation was established for the Gyr ($r = 0.3279$), Jersey ($r = 0.2759$) and Holstein (NR) ($r = 0.0478$) cows.

A negative correlation was observed between average tick infestation and the number of mast cells in the upper ($r = -0.21$, $P <0.01$) and deep dermis ($r = -0.021$, $P>0.05$) and the total number of dermal mast cells ($r = -0.1336$, $P>0.05$) - taking into account all the data collected – irrespective of the breed, indicating that the greater the number of these cells in the skin - mainly in the upper layer of the dermis - the less intense the tick infestation.

Discussion

Zebu cows of the Nelore breed had the largest number of mast cells in the dermis as compared with the other breeds investigated in this study. It is well known that this breed has great resistance towards the *R. microplus* tick (6, 25). On the other hand, the Gyr Zebu breed exhibited a lower number of dermal mast cells when compared to the European breeds, such as Holstein (R) and Brown-Swiss. This fact is in disagreement with Lavielle et al. (9), Moraes et al. (12) and Sartor et al. (18), who found that Zebu cows had a larger number of mast cells than European or crossbred cows.

There are no data available in literature on the number of dermal mast cells in Nelore, Brown-Swiss and Jersey cows.

Moraes et al. (12) found averages of 43.1 and 110.2 mast cells/mm2 in the groin skin of five 20-month-old Holstein and Gyr calves, respectively. These numbers are similar to those found in the present study for mast cells/mm2 in the inner dermis of Holstein cows (NR) (48.76) and the total number of mast cells in the Gyr breed (100.98).

Studies by Sartor et al. (18) carried out on 15 to 20 month-old Gyr, F1 crossbred and Holstein calves, demonstrated that 24 hours after artificial infestation with larvae of *R. microplus*, the animals presented about 100, 40 and 30 dermal mast cells/mm2, respectively. The authors found a positive correlation between the presence of mast cells and the level of resistance of the animals to the tick; therefore the greater the number of these cells in the dermis, the smaller the number of ticks on the animals.

Engracia Filho et al. (3) determined the number of dermal mast cells in 148 F2 Holstein x Gyr crossbred cattle before and after artificial infestation with 10,000 *R. microplus* larvae per animal. The infestation induced a significant increase in the number of mast cells in the upper dermis of the cattle, and a negative and significant correlation ($r = -0.2005$, $P<0.0167$) was found with the intensity of tick infestation. There was no significant correlation between the number of ticks and the number of mast cells in the upper, deep and total dermis before artificial infestation. The authors also observed a positive and significant correlation between the age of the cattle (1 to 2 year-old) and the number of mast cells in the upper, deep and total dermis, suggesting that the oldest animals had a greater number of mast cells in the skin.

Since in the present study infestation was not controlled at the moment of biopsy, and taking into consideration the fact that the Holstein (R) and Brown-Swiss cows were subjected to repeated high natural infestations by *R. microplus*, the large number of mast cells found in these animals could be explained by the high exposure to tick larvae. It should be stressed that biopsies were taken in the spring, a time of year favorable for the non-parasitic phase of the cattle tick. Table 1 clearly shows that the Holstein (R) and Brown-Swiss with the smallest

Cattle breed	Low infestation					High infestation				
	N	*R. microplus*	MCup	MCdp	MCtot	N	*R. microplus*	MCup	MCdp	MCtot
Nelore	15	0.0 ± 0.0	145.51 ±62.38	99.28 ±53.58	244.79 ±103.63	14	20.78 ±8.74	135.80 ±54.95	82.45 ±57.78	218.25 ±98.52
Holstein (registered)	11	32.29 ±14.22	83.61 ±31.88	93.71 ±44.24	177.33 ±67.90	10	193.2 ±112.4	53.70 ±27.15	69.14 ±40.72	122.84 ±54.63
Brown-swiss	11	26.86 ±16.98	83.61 ±45.39	93.15 ±71.75	176.76 ±91.69	10	155.62 ±91.27	49.38 ±24.34	67.28 ±58.66	116.66 ±73.99
Gyr	13	2.52 ±1.43	56.98 ±44.31	34.66 ±16.04	91.64 48.90	12	15.06 ±5.64	66.87 ±34.70	44.24 ±29.17	111.10 ±57.49
Holstein (non-registered)	10	60.7 ±21.67	34.57 ±28.84	34.57 ±28.69	69.14 ±51.22	10	175.52 ±100.72	62.96 ±61.44	52.47 ±35.67	115.43 ±91.42
Crossbred	5	12.00 ±3.86	19.75 ±30.68	17.28 ±21.11	37.03 ±47.41	6	38.75 ±14.95	56.58 ±90.84	78.19 ±130.52	134.77 ±220.17
Jersey	15	2.37 ±1.40	27.16 ±21.61	33.33 ±22.35	60.49 ±41.03	13	104.96 ±50.09	31.81 ±26.77	51.28 ±27.18	83.09 ±45.19

Table 1 – Counts of the cattle tick *Rhipicephalus (Boophilus) microplus* and mast cells in the upper (MCup), deep (MCdp) and total (MCtot) dermis of European, Zebu and crossbred cows classified as bearing high or low tick infestation. Results are expressed as mean ± standard deviation, N= number of animals.

Breeds	N	Number of upper dermal mast cells	Number of deep dermal mast cells	Total number of dermal mast cells
Nelore	29	139.42 a	90.58 a	230.00 a
Holstein (registered)	21	69.37 b	82.01 ab	151.38 b
Brown-swiss	21	67.31 bc	80.83 ab	148.14 b
Gyr	25	61.73 bc	39.25 b	100.98 bc
Holstein (non registered)	20	48.76 bc	43.51 b	92.28 bc
Crossbred	11	39.84 bc	50.50 ab	90.34 bc
Jersey	28	29.32 c	41.66 b	70.98 c
General average	155	69.43 (3.79)	62.25 (3.75)	131.67 (4.56)
Cv%		64.26	78.76	63.17

Averages followed by different letters in the columns statistically differ among each other

Table 2 – Mast cell counts (cells/mm^2) in the upper, deep and total dermis of different breeds of European, Zebu and crossbred cows.

Cattle breeds	N	Upper dermal mast cells (r)	Deep dermal mast cells (r)	Total dermal mast cells (r)
Nelore	29	- 0.14640 n.s.	- 0.25898 n.s.	- 0.22759 n.s.
Holstein (r)	21	- 0.33672 n.s.	- 0.04373 n.s.	- 0.1944 n.s.
Brown-swiss	21	- 0.32319 n.s.	- 0.30845 n.s.	- 0.38009 n.s.
Gyr	25	0.18823 n.s.	0.42244 *	0.32790 n.s.
Holstein (nr)	20	- 0.01995 n.s.	0.14036 n.s.	0.04780 n.s.
Crossbred	11	- 0.13544 n.s.	- 0.0369 n.s.	- 0.07859 n.s.
Jersey	28	0.08671 n.s.	0.38620 *	0.27590 n.s.
All animals	155	- 0.21087 **	- 0.02104 n.s.	- 0.13364 n.s.

Table 3 – Correlation (r) between the number of mast cells and ticks in different breeds of European, Zebu and crossbred cows. ** P < 0.01; * P < 0.05; n.s. - non-significant

number of tick infestations also exhibited a larger number of mast cells in the dermis.

The differences in sex, age, genetics and intensity of tick infestation between animals as reported by Sartor

(18), Moraes et al. (12) and in the present study could explain the variability in the average mast cell counts in the dermis of the Holstein breed.

Observations relative to Holstein cows of other herds, in addition to the mobilization dynamics of mast cells in the skin as influenced by the age of the animals, indicates the need of conducting more detailed study on the evolution of the number of mast cells in the skin of that particular breed.

Veríssimo et al. (27) performed tick counts on the total Jersey herd investigated in this study and found that more than 70% of the animals pertained to the class of animals categorized as "tick-resistant" (up to 25 ticks larger than 4.0mm, counted on the right side). However, in the sample animals of this herd, the smallest number of mast cells was found in the dermis. Table 1 shows that the average number of ticks on the Jersey cows with low infestation intensity was 2.37, much lower than the average number of ticks found on the Holstein (NR) (60.7), Holstein (R) (32.29) and Brown-Swiss (26.86) cows of the same category.

A study on the presence of other cells, such as eosinophils and basophils is required to better explain the resistance mechanism of Jersey cows to the larvae of the cattle tick *R. microplus*.

The correlation between the number of mast cells in the upper dermis and the number of ticks was negative and highly significant ($r = -0.21087$, $P < 0.01$). In other words, the larger the number of mast cells in the upper dermis, the smaller tick infestation. This was also observed by Engracia Filho et al. (3) and Sartor et al. (18). Gill (4), who studied cell reactions associated with acquired resistance of cattle to the *Hyalomma anatolicum* tick and suggested that the chemical mediators released by degranulated mast cells and basophils play an important role in the resistance mechanism of cattle to this tick species. In fact, there seems to be a direct relationship between mast cells and the chemical mediators they secrete and store, mainly histamine, and host rejection of *R. microplus* larvae.

Several authors (2, 8, 15, 21) demonstrated that self-grooming is extremely important to decrease the number of *R. microplus* larvae that stick to the host, and associated this phenomenon with the release of histamine by degranulated mast cells in the area where the larvae attach. In fact, when antihistamines are administered to the animals - Taurine or Zebu cattle - tick infestation increases (22). Therefore, the magnitude of the mast cells' number in the site of the biopsy herein determined could be affected by the dermal mast cell influx due to the cellular degranulation degree produced by the tick bites.

Kemp and Bourne (7) observed in both "in vivo" and "in vitro" experiments that, of all the pharmacological mediators released in an inflammatory reaction, only histamine acted on the larvae in order to keep them from feeding and cause them to loosen from the host's skin. The authors suggested that histamine has a direct effect on tick larvae. Actually, mast cells seem to be an important factor in the development of resistance of other hosts to other parasites, such as swine to nematoids (13) and sheep to *Haemonchus contortus* (5, 14).

Matsuda et al. (10) demonstrated the importance of mast cells in the mechanisms of host resistance to the *Haemaphysalis longicornis* tick. The authors studied mast cell- deficient and normal mice and found that mast cell-deficient mice were not capable of expressing resistance to the ectoparasite, unlike the normal mice. Such resistance was restored in mast cell-deficient mice that received a bone marrow transplant from normal mice, a procedure that resulted in mast cell differentiation and multiplication in the skin of the deficient mice. The introduction of mast cells directly into the skin of deficient mice lead to the development of resistance to the ixodid, but only in the inoculated skin areas, with the growth of new mast cells (11).

Taking these data into consideration, our results support the hypothesis that dermal mast cells play an important role in the mechanism of resistance to the cattle tick *R. microplus*.

Moreover, a study by Veríssimo (24) on the resistance mechanisms of cattle to the *R. microplus* tick leads to the conclusion that the immediate hypersensitivity mechanism is very important, as it determines the resistance of the host to that particular tick species. According to this study, histamine is distributed throughout a mammal's organism, especially in cytoplasmic granules of the connective tissue mast cells and in circulating leukocytes. When released from their storages, they can cause reactions that go from a simple itch to circulatory shock and death. The results obtained by Willadsen et al. (28) suggest that the main pharmacologically active agent in cutaneous hypersensitivity reactions to *R. microplus* larvae is histamine, and that the total amount of it available locally in the skin may have a crucial role in the resistance to this parasite. In addition, Bechara et al. (1), using the hypersensitivity skin test in Holstein and Nelore calves demonstrated an immediate type response to unfed larval extract of *R. microplus* in both the breeds, while a delayed type response was observed only in the latter. The authors concluded that the cellular arm of the immune response may play an important role in the resistance mechanism of cattle to the *R. microplus* tick.

The great number of mast cells found in both the upper and deep dermis of Nelore cows could explain their marked resistance to the *R. microplus* tick. However, the presence of other cells that infiltrate the inflammatory response to the larvae, such as eosinophilis and basophils, also important in the resistance mechanism of taurines to the tick (19, 20), should be investigated, so as to explain this mechanism of the Jersey breed to the cattle tick.

The great number of mast cells found in the dermis of Holstein and Brown-Swiss cows indicate that they could carry out efficient self-grooming, however other

mechanisms must be involved to explain the great differences in terms of degree of resistance to the *R. microplus* tick between these European and Zebu breeds.

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