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Resistance of cattle of various genetic groups to the tick *Rhipicephalus microplus* and the relationship with coat traits

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ABSTRACT

This study evaluated the resistance of cattle of different genetic groups to the tick *Rhipicephalus microplus* and the relationship with traits of the animals' hair and coat. Cows of the Senepol × Nelore (SN), Angus × Nelore (AN) and Nelore (NX) genetic groups were submitted to four consecutive artificial infestations, at 14-day intervals, each one with approximately 20,000 tick larvae placed on the animals' lumbar region. From the 19th to 23rd day of each infestation five counts of the number of ticks were performed on each animal's left body side. The tick count data (TTC) were transformed into $\log_{10}(n+1)$, and also into percentage of return (PR), where n is the total number of ticks counted at each infestation. Hair samples were collected 24 h after the last infestation with flat-nosed pliers. Measures of the average hair length (HL), coat thickness (CT), number of hairs per cm^2 (NHCM2) and weight of the samples (SW) were obtained. Pearson's correlation coefficients were calculated within genetic group to measure association between PR and the hair and coat data. There was a significant difference among genetic groups for the number of ticks, with the AN group having higher counts than the SN and NX groups. For the hair and coat traits, the NX and SN groups had lower values of HL and SW than did the AN group. The SN genetic group had lower NHCM2 counts than the NX and AN groups. There were positive correlations between TTC and CT ($P<0.05$) and SW ($P<0.05$) in the SN group. No significant correlation was found for the AN genetic group ($P>0.05$).

1. Introduction

Infestations by ectoparasites are among the main problems that affect stock raising in tropical countries (Jonsson, 2006; Bianchin et al., 2007). Among these parasites, the cattle tick *Rhipicephalus microplus* stands out, causing weight loss, anemia and skin lesions, as well as transmitting diseases such as babesiosis and anaplasmosis to herds (Jongejan and Uilenberg, 2004; Jonsson, 2006; Chanie et al., 2010).

The application of acaricides is the most common method used to control cattle ticks. However, the improper use of these chemicals compounds has been causing the development of tick resistance to various pesticides available in the market, reducing these products' useful lifetimes. Besides this, problems generated by the presence of chemical residues in meat, milk and the environment have prompted reflection on the need for better monitoring of their application (Graf et al., 2005; Castro-Janer et al., 2010). Therefore, the study of the genetic resistance

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to ticks among different breeds of cattle can contribute to the development of alternative control methods (Sutherst and Utech, 1981; Gasparin et al., 2007).

It is widely known that *Bos indicus* cattle are more resistant to ectoparasites than are *Bos taurus* animals (Bianchin et al., 2007). Studies are intensifying the crossing of these two groups, aiming to obtain animals that are more resistant to the conditions found in tropical countries and are also good meat producers (Frisch et al., 2000; Oliveira et al., 2009).

Silva et al. (2007) found that Nelore cattle have greater resistance to ticks than do Canchim × Nelore crosses, followed by Angus × Nelore and Simental × Nelore. Similar patterns in relation to increased resistance to ticks with higher *Bos indicus* concentration have also been observed by Utech and Wharton (1982), Wambura et al. (1998) and Singh and Ghosh (2003).

Tick resistance among cattle is influenced by a number of factors. The most important are increased levels of histamine at the early stages of the infestation (Kemp and Bourne, 1980), self-cleaning behavior (De Castro et al., 1985), increased levels of eosinophils, basophils and mast cells (De castro and Newson, 1993), the presence of specific immunoglobulin patterns (Kashino et al., 2005), T cells (Piper et al., 2010) and genes related to the expression of keratins and lipocalins (Kongsuwan et al., 2010). Traits of the hair and coat also can be related to the severity of tick infestation, but there is little data available on the relationship of these traits with tick resistance. Longer hairs, thicker coats and greater number of hairs have been reported as associated with increased tick infestation (Spickett et al., 1989; Veríssimo et al., 2002; Gasparin et al., 2007). However, the findings of these research teams differ from those reported by Burns et al. (1988), who found no relation between these traits and tick resistance. Hence, there is a need for further studies.

Investigation of crossbreeding is important to verify the advantages of heterosis, both in relation to productive and adaptive traits. Although the Nelore breed is recognized as being resistant to cattle ticks, little information is available on crosses with this breed. Besides this, little is known relative to the influence of coat traits on resistance to the cattle tick *R. microplus*. Therefore, the aim of this study was to evaluate the degree of resistance of cattle of three genetic groups: Nelore (NX), ½ Nelore + ½ Senepol (SN) and ½ Nelore + ½ Angus (AN) to the cattle tick *R. microplus*, and its relation to the traits of these animals' hairs and coats.

2. Materials and methods

2.1. Animals

The study was performed on the experimental farm of Embrapa cattle-Southeast, located in the municipality of São Carlos, São Paulo state, Brazil (latitude 22°1′S, longitude 47°53′W and 856 m altitude). We used 69 heifers, 25 from each genetic group ½ Senepol + ½ Nelore and purebred Nelore and 19 from ½ Angus + ½ Nelore group. The experimental animals were born from November 2005 to January 2006. During the suckling period, the animals were maintained in Tanzania grass pastures. The Nelore (*B. indicus* breed) is considered the best adapted to the prevailing conditions in Brazil. The two *B. taurus* breeds were used in this experiment because they show different degrees of adaptation. The Senepol breed originated from crossing the N'Dama breed of the Sanga group, which is extremely resistant to parasites (Mattioli et al., 1993) to Criollo cattle and with the British Red Poll breed, while Angus is the *B. taurus* breed most often used in crossbreeding for beef production in Brazil and is highly susceptible to tropical parasites. The females of the three groups were produced by artificial insemination of Nelore cows of the same genetic base with semen from five, five and eight bulls of the Nelore, Senepol and Angus breed, respectively.

2.2. Artificial infestations

Before the start of the experiment, the animals were treated with a commercial acaricide containing amitraz (Triatox®). The artificial infestations with *R. microplus* larvae were started 30 days later, after which the animals did not receive any further acaricides until the end of the experiment. To obtain the infective larvae, only engorged *R. microplus* females with weights ranging from 160 to 300 mg were selected, because they are considered optimal for oviposition (Bennett, 1974). The female ticks were washed and placed in sterile petri dishes and incubated in a BOD at 27 ± 1 °C and relative humidity above 80% for oviposition (15 days). The eggs were then weighed in aliquots of 1 g, each containing about 20,000 eggs (Gonzales, 1993), and placed in 20-mL syringes. The syringes containing the eggs were incubated again in the BOD under the same temperature and humidity conditions described above, until hatching of the larvae (14 days). Only the syringes that showed hatching rates above 90% by visual inspection were used. There were four successive artificial infestations between March 5 and April 16, 2008, at 14-day intervals, using larvae between 15 and 21 days old. The animals were infested by securing them in a squeeze chute and emptying the entire contents of a syringe along the animal's lumbar region. On the 19th through the 23rd day after each infestation, we performed five counts (one per day) of the number of engorged females that measured at least 4.5 mm in diameter present on the left side of each animal. During the experiment the average air temperature was 22.15 °C, ranging from 16.8 °C to 27.6 °C, with relative air humidity averaging 72.6%.

2.3. Measurement of coat thickness and collection of hair samples

During the last infestation, the coat thickness (CT) was measured with a metal millimeter ruler with a cursor attached. The ruler was inserted vertically in the fur until touching the epidermis and then the cursor was moved until reaching the coat surface (Silva, 2000). After measurement of the coat thickness, hair samples were obtained from the same spot, with a pair of flat-nose pliers with a spreader, so that when the pliers were pressed to close them, the jaws were kept 1.5 mm apart. To pull out the hairs, the pliers were introduced at a right angle in relation to the epidermis and moved in a combing motion while touching the epidermis. Then the spreader was removed to allow the pliers to grab the hairs, which were pulled out firmly. The hair sample from each animal was placed in a small plastic bag marked in advance with the animal's number and the following data were recorded: number of hairs per cm^2 (NHCM2), average hair length (HL) and weight of the sample (SW), according to Silva (2000).

2.4. Statistical analysis

The data from the artificial infestations were analyzed in terms of total tick count (TTC) transformed to $\log_{10}(n+1)$ and percentage of return (PR), that is, the percentage of ticks counted on one side in relation to the total larvae infested, calculated by $PR_{ij} = 400C_{ij}/20{,}000$, where: 400 results from the multiplication 100 (percentage) × 2 tick sex (males and females) × 2 sides of the animal (right and left), i = is the heifer, j is the infestation number ($j = 1, \ldots, 4$), 20,000 is the number of tick larvae used for the each infestation and C_{ij} is the sum of the number of ticks counted on heifer i, over the five count days (19th through the 23rd day after the infestation j). For statistical analysis of the data, PR_{ij} was transformed to $PRT_{ij} = PR_{ij}^{1/4}$, as suggested by Oliveira and Alencar (1987). The data (PRT and TTC) were then subjected to repeated measure analysis by the MIXED procedure of the SAS program, with a model that included the fixed effects of genetic group (GG), infestation (IN) and their interactions, besides the residual. A compound symmetry structure was assumed for the within-subject (animal) variance–covariance matrix. The tick mortality was measured by subtracting the percentage of return from 100. The genetic groups were classified in resistance levels according Utech et al. (1978).

The data on each hair trait – number of hairs per cm^2 (NHCM2), average hair length (HL), hair sample weight (SW) and coat thickness (CT) were submitted to analysis of variance with a model that included the fixed effect of genetic group (GG), utilizing the GLM procedure of the SAS program. Tukey Kramer adjustment for multiple comparisons was used to compare the means between the genetic groups.

To analyze the associations of the traits NHCM2, HL, SW, CT and TTC (average of the four infestations) these variables were submitted within genetic group to Pearson's correlation analysis. All the statistical analyzes were performed using the SAS statistical package (SAS, 2002/2003).

3. Results

3.1. Tick counts

Means and standard errors of untransformed data (TC) for NX, SN and AN were, respectively, 8.52 ± 7.26; 18.81 ± 7.26 and 75.34 ± 8.33. There was a significant effect of the genetic group ($P < 0.05$) on the tick counting numbers (TTC), with means and standard errors presented in Table 1. Results were similar for the percentage of return (PRT) (Table 1). For the untransformed data (PR) the means and standard errors were $0.17\% \pm 0.14$ for NX; $0.38\% \pm 0.14$ for SN and $1.51\% \pm 0.17$ for AN. No significant interaction between genetic group and infestation number was detected either for TTC or PRT (Table 1). In the three studied genetic groups tick counts increased according to infestation number (Fig. 1). This occurs because besides that artificial infestation, the animals could be naturally infested in pastures, influencing in the animals tick counts, as showed by Silva et al. (2007).

3.2. Hair and coat analyses

In relation to the effect of genetic group on the hair and coat traits, the AN group had higher HL and SW values ($P < 0.01$) than did the NX and SN animals. The SN animals presented lower values of NHCM2 ($P < 0.05$) than did those from the AN and NX groups, and the last two groups did not differ from each other. For the CT measure, the NX animals did not differ from the SN and AN animals ($P > 0.05$), but there were differences between the SN and AN animals ($P < 0.05$, Table 2).

3.3. Correlation analysis

The correlations obtained for the hair, coat and percentage of transformed tick count (TTC) are presented in Table 3. The SN genetic group showed a positive correlation between TTC and CT (0.51; $P < 0.05$), and between TTC and SW (0.52; $P < 0.05$). For the NX animals, there was a positive correlation of the NHCM2 variable with CT (0.42; $P < 0.05$). Finally, for the AN genetic group there was no significant correlation between these variables ($P > 0.05$).

4. Discussion

The utilization of crossbreeding cattle has been shown to be an effective alternative to control ectoparasites in countries such as Australia (Sutherst and Utech, 1981) and is currently attracting great interest from stock breeders in Brazil to improve the productive efficiency of herds. In this study we evaluated the variation in resistance of purebred Nelore cattle and Senepol × Nelore and Angus × Nelore crosses and investigated the relationship with the coat traits. The animals of the NX group were infested by fewer ticks (Fig. 1), but no significant differences were found between this and the SN group, so both were considered the most resistant. The Nelore breed has been identified as more resistant to ticks than taurine breeds, both in studies with natural and artificial infestations (Silva et al., 2007, 2010). Besides those differences in the tick counts observed, in our work, the three groups had more than 98% of tick mortality, indicating high resistance level to tick according to Utech et al. (1978) classification.

The SN group had no differences in number of ticks and percentage of return as compared to the NX group, its averages reveal animals with high resistance, mainly in comparison with the AN group, that have more tick counts (Table 1). Animals of the Senepol breed are considered very resistant to various parasites, as helminthes and trypanosomosis (Mattioli et al., 1993; Oliveira et al., 2009) and are also known for being heat tolerant (Hammond and Olson, 1994; Ribeiro et al., 2009). Here we showed, for the

Table 1
Estimated means of the $\log_{10}$ $(n+1)$ of tick counts (TTC) and transformed percentage of return (PRT) according to genetic group (NX = Nelore, SN = Senepol × Nelore and AN = Angus × Nelore), and infestation.

Cattle genetic group*	TTC				
	Infestation*				Overall
	1	2	3	4	
NX	0.40 ± 0.09	0.50 ± 0.09	0.80 ± 0.09	1.00 ± 0.09	0.68 ± 0.08^{A}
SN	0.62 ± 0.09	0.70 ± 0.09	1.07 ± 0.09	1.31 ± 0.09	0.93 ± 0.08^{A}
AN	1.26 ± 0.10	1.44 ± 0.10	1.83 ± 0.10	2.00 ± 0.10	1.63 ± 0.09^{B}
Overall	0.76 ± 0.05^{a}	0.88 ± 0.05^{b}	1.23 ± 0.05^{c}	1.43 ± 0.05^{d}	1.08
Cattle genetic group*	**PRT**				
	Infestation				Overall
	1	2	3	4	
NX	0.36 ± 0.05	0.39 ± 0.05	0.55 ± 0.05	0.65 ± 0.05	0.49 ± 0.04^{A}
SN	0.45 ± 0.05	0.51 ± 0.05	0.69 ± 0.05	0.82 ± 0.05	0.62 ± 0.04^{A}
AN	0.79 ± 0.06	0.86 ± 0.06	1.09 ± 0.06	1.22 ± 0.06	0.99 ± 0.05^{B}
Overall	0.53 ± 0.03^{a}	0.59 ± 0.03^{a}	0.77 ± 0.03^{b}	0.90 ± 0.03^{c}	0.7

* Different upper-case letter in the same column indicate difference for genetic group and different lowercase letters in the same line indicate a significant difference for infestation. ($P \leq 0.05$).

Table 2
Estimated means of hair and coat traits: average hair length (HL), coat thickness (CT), number of hairs per cm^2 (NHCM2) and hair sample weight (SW), according to the genetic group (NX = Nelore, SN = Senepol × Nelore and AN = Angus × Nelore).

GG	HL (mm)	CT (cm)	NHCM2 (hair/cm^2)	SW (g/cm^2)
NX	10.21 ± 0.48^{a}	0.40 ± 0.04^{a,b}	1453.20 ± 83.52^{a}	0.0020 ± 0.0002^{a}
SN	11.24 ± 0.50^{a}	0.32 ± 0.04^{a}	1080.27 ± 85.32^{b}	0.0021 ± 0.0002^{a}
AN	15.41 ± 0.54^{b}	0.50 ± 0.04^{b}	1406.07 ± 93.88^{a}	0.0032 ± 0.0002^{b}

[a,b] Means with the same letters within columns are not significantly different ($P < 0.05$).

first time, that Nelore × Senepol crosses produce animals as resistant as pure Nelore to *R. microplus* tick. In this way, animals of this breed and its crosses acquire traits such as parasite resistance and heat tolerance from their *B. indicus* ancestry, as well as retaining the high productivity of their *B. taurus* ancestry (Hammond and Olson, 1994; O'Connor et al., 1997; Ribeiro et al., 2009; Oliveira et al., 2009), making them an alternative for production in tropical countries

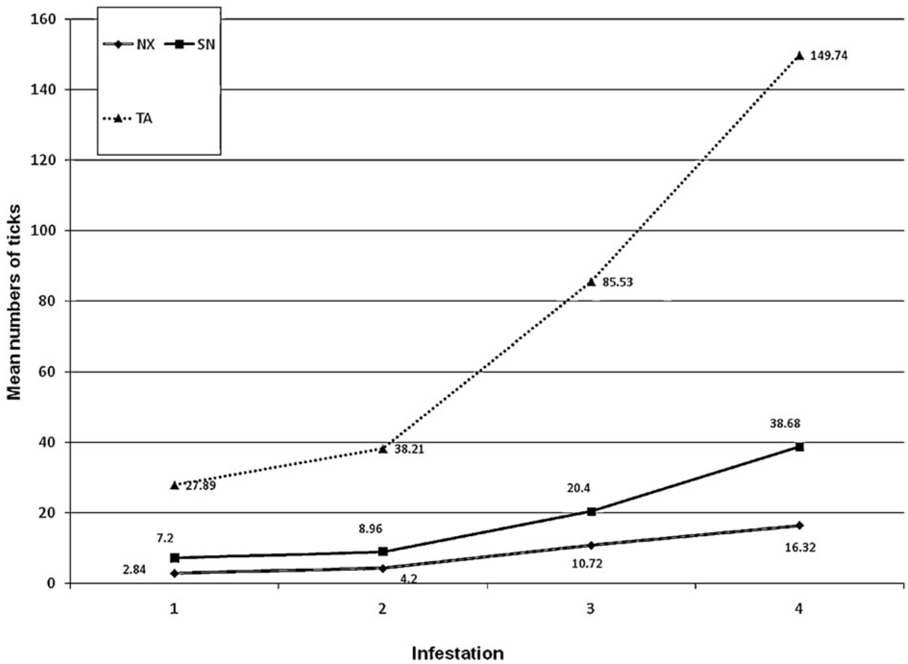

Fig. 1. Mean number of counted ticks according to infestation and genetic group.

Table 3
Pearson's correlation analysis of the transformed tick counts (TTC) with hair and coat traits, according genetic group (NX = Nelore, SN = Senepol × Nelore and AN = Angus × Nelore).

	Genetic Group		
	NX	SN	AN
Average hair length (HL)	0.006^{ns}	-0.050^{ns}	-0.076^{ns}
Coat thickness (CT)	0.067^{ns}	0.506^{*}	0.127^{ns}
Number of hairs per cm^2 (NHCM2)	0.423^{*}	-0.023^{ns}	0.017^{ns}
Hair sample weight (SW)	0.059^{ns}	0.517^{*}	0.190^{ns}

ns = non-significative.
* $P < 0.05$.

like Brazil. The skin and coat traits, along with the hide color, can be related to maintenance of ticks on the host (O'Kelly and Spiers, 1983), but these traits have not been widely studied. In this work it was possible to observe that the AN group, with the highest number of ticks, also had the greatest hair lengths and sample weights in comparison with the NX and SN animals (Table 2). In contrast, the SN group had the lowest coat thickness and number of hairs per sample in comparison with the other two groups studied (Table 2) but had tick counts similar to Nelore. The correlation analysis, however, showed that the traits that could influence the animals' resistance to ticks varied with the genetic group. In the NX group there was a tendency for the animals with a greater number of hairs per area to have more severe infestations (Table 3), as was also observed by Veríssimo et al. (2002) in purebred and crossbred Gyr. For the SN group, both the coat thickness (CT) and sample weight (SW) were positively correlated with the tick infestation (Table 3). Fraga et al. (2003), also found that animals with thinner coats and lower hair sample weights tend to be less infested by ticks. According to the authors of this study, cattle with longer hairs and thicker coats create a microclimate that is more favorable to tick survival. Beside these factors, heat stress has been shown to influence resistance to tick infestation. When cattle are subjected to heat stress, the levels of glucocorticoids in their bloodstream can increase, affecting the antiinflammatory and antiallergic effects, possibly favoring the presence of ticks (Fraga et al., 2003).

In this study, although the AN group presented a greater number of hairs and longer hairs than the other two groups, there was no correlation between these traits and the number of ticks infesting them. These results differ from other reports in the literature, since in other studies with cattle of European breeds and crossbreeds a positive correlation was found between these two coat traits and the number of ticks (O'Kelly and Spiers, 1983; Spickett et al., 1989). This shows that coat traits can influence in different levels the tick resistance. In that case, other factors could be more important to confer susceptibility, as coat color, thermotolerance or even another genetic traits (immune system, coagulation factors and MHC complex).

According to our data, it is possible to conclude that NX and SN genetic groups are highly resistant to *R. microplus* tick, being a choice for beef cattle production in the tropics while avoiding the severe problems related to this parasite. Coat traits influenced resistance to these two genetic groups. Moreover, as the resistance to ticks in NX and SN animals are similar, the use of SN animals can be an alternative to increase the productivity in crossbreeding systems without increasing the use of acaricides.

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