Genetics and Molecular Biology, 30, 4, 1150-1155 (2007)

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Research Article

Artificial infestation of *Boophilus microplus* in beef cattle heifers of four genetic groups

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Abstract

Resistance of beef cattle heifers to the cattle tick *Boophilus microplus* was evaluated by artificial infestation of 66 beef cattle heifers of the following genetic groups: 16 Nelore (NE), 18 Canchim x Nelore (CN), 16 Angus x Nelore (AN) and 16 Simmental x Nelore (SN). The animals, with a mean age of 16.5 months, were maintained with no chemical tick control in a *Brachiaria decumbens* pasture. Four artificial infestations with 20,000 *B. microplus* larvae were carried out 14 days apart and from day 18 to day 22 of each infestation the number of engorged female ticks (≥ 4.5 mm) was counted on the left side of each heifer. Data were analyzed as the percentage of return (PR = percentage of ticks counted relative to the number infested), transformed to $(PR)^{1/4}$, and as $\log_{10}(C_{ij} + 1)$, in which C_{ij} is the number of ticks in each infestation, using the least squares method with a model that included the effects of genetic group (GG), animal within GG (error a), infestation number (I), GG x I and the residual (error b). Results indicated a significant GG x I interaction, because AN and SN heifers had a higher percentage of return than CN and NE heifers, while CN heifers showed a higher percentage of return than the NE heifers only in infestations 3 and 4. Transformed percentages of return were NE = 0.35 ± 0.06, AN = 0.89 ± 0.06, CN = 0.54 ± 0.05 and SN = 0.85 ± 0.06.

Key words: beef cattle, crossbred, tick resistance.

Received: May 16, 2006; Accepted: January 22, 2007.

Introduction

The cattle tick *Boophilus microplus* is an external parasite present in tropical and subtropical areas of America, Africa, Asia and Australia (Leal *et al.*, 2003). Tick parasitism is one of the most detrimental environmental factors affecting cattle production and performance because it causes immunosuppression in the affected cattle (Jonsson, 2006). In both beef and dairy herds the main damage caused by cattle ticks are the costs involved with chemical products and equipment used for parasite control along with losses in fertility, body weight and milk production, although other important losses include leather depreciation due to tick puncture marks and the transmission of infectious diseases, principally *Anaplasma and Babesia* (Seifert *et al.*, 1968; Gugliemone, 1995; Wambura *et al.*, 1998; Gonçalves *et. al.*, 1999). Furthermore, the indiscriminate use of chemical products may affect future parasite control as a consequence of the development of resistance to the active principle used for tick control preparations (Fraga *et al.*, 2003).

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There are great differences between *Bos indicus* (Asian) and *Bos taurus* (European) cattle in regard to their susceptibility to parasitism by cattle ticks, the scientific literature reporting that infestation increases as the proportion of European genes in an animal increases (Lemos *et al.*, 1985). Studies show that, in general, the number of ticks on zebu (*B. indicus*) cattle and their crossbreds (zebu x European) is significantly less than the number found on European breeds (Johnston and Haydock, 1969; O' Kelly and Spiers, 1976; Utech and Wharton, 1982). In Brazil, several workers have also reported different degrees of tick-resistance in cattle, both among and within breeds (Lemos *et al.*, 1985; Oliveira *et al.*, 1989; Oliveira and Alencar, 1990; Fraga *et al.*, 2003). These breed differences can be used to match the genotype of the animal to their environment and increase the productive efficiency of herds, thereby satisfying the demands of consumers for high-quality products and respect for the environment (Alencar *et al.*, 2005). In fact, crossing *B. taurus* and *B. indicus* breeds has been used in Brazil to rapidly increase the productivity of beef cattle systems, producing adapted cattle of high potential as a consequence of heterosis and complementarity. Therefore, it is necessary to characterize the different crossbreeding systems so that producers can make the correct decisions when choosing the breeds and crossbreeding system.

The objective of the study reported in this paper was to evaluate the degree of tick-resistance of beef heifers of different genetic groups when artificially infested with the cattle tick *Boophilus microplus*, this study being part of a program of characterization and evaluation of crossbreeding systems.

Material and Methods

Animals

This study was undertaken at the Brazilian Agricultural Research Corporation (*Empresa Brasileira de Pesquisa Agropecuária* - Embrapa), Southeast – Embrapa Cattle (SEC) unit, located at 22°01’ S, 47°53’ W near the city of São Carlos in the Brazilian state of São Paulo. The climate of the region is tropical CAw on the Köppen climate classification and in the last 13 years the coldest months were June and July at 18.3 °C, the warmest was February at 23.6 °C, the driest was August with a rainfall of 20 mm and the wettest was January with a rainfall of 256 mm. These values represent the average values over 13 years.

The heifers investigated were 16 Nelore, 18 Canchim x Nelore, 16 Angus x Nelore and 16 Simmental x Nelore. These breeds were chosen to participate in a crossbreeding research project because the aim was to produce offspring different in production potential and in environmental adaptive capacity. Nelore (*B. indicus*) is a white-coated breed which is the most widely raised beef-breed in Brazil. Canchim (5/8 Charolais + 3/8 zebu) is a cream-coated synthetic breed formed in Brazil; Angus (*B. taurus*) is a black-coated British European breed; and Simmental (*B. taurus*) is a cream-coated or white and yellow-coated continental European breed. Heifers of all four genetic groups had the same Nelore genetic basis in that they were offspring of Nelore or high grade Nelore dams of the same origin, dams and heifers being maintained on Tanzania grass (*Panicum maximum cv Tanzania*) pastures up to weaning. The heifers were sired by three Nelore and three Canchim bulls, by natural service, and nine Angus and seven Simmental bulls, by artificial insemination. At the beginning of the experiment, females, born from August to November of 2003, were, on average, 16.5 months old, and were kept in a Brachiaria grass (*Brachiaria decumbens*) pasture without any kind of tick control.

Ticks and infestation

Engorged adult female *Boophilus microplus* ticks were collected from naturally infested cattle at SEC and incubated in a biological oxygen demand (BOD) chamber at 27 °C ± 1 °C and a humidity of at least 85%-86% to produce eggs. The eggs were harvested from the female ticks on the 15^{th} day of incubation and 1 g (about 20,000 larvae) aliquots placed in flasks and returned to the BOD chamber under the same conditions until hatching. Only flasks in which over 90% hatching occurred, by visual examination, were used for infestation. All larvae used for infestation were from 15 to 20 days old.

Each heifer was artificially infested with 20,000 larvae on four separate occasions 14 days apart (13 and 27 of January and 10 and 24 of February 2005) by emptying the contents of one flask on the back of each heifer. On the first infestation we counted the number of engorged female ticks (≥ 4.5 mm) on the left side of each heifer from day 20 to day 22 (three counts), while for the subsequent three infestations the counts were made on days 18 to 22 of each infestation (five counts). The reason why only three counts were made in the first infestation was that it was not possible to do the first two counts. During the experimental period, average daily mean temperature was 23.25 °C (minimum 18.25 °C, maximum of 28.2 °C), average daily relative humidity was 85% and average daily rainfall was 7 mm. At the end of the experiment the heifers were treated with an acaricide.

Statistical analysis

Data from the artificial infestations were analyzed as the percentage of return (PR) as given by the percentage of ticks counted relative to the number infested on one side of the heifer for a tick sex ratio of 1:1 male:female, *i.e.* $PR_{ij} = 400C_{ij}/20{,}000$, where i is the heifer, j is the number of the infestation (1,..., 4), 400 results from 100 (percentage) x 2 (two tick sexes) x 2 (two sides of the animal), $C_{ij} = \Sigma C_{ijk}$ (where ΣC_{ijk} is the sum of the number of ticks counted (C), i and j are as above and k is the count number (1,..., 5)), and 20,000 is the number of tick larvae used for each infestation. For analysis, PR_{ij} was transformed (T) to $PRT_{ij} = (PR_{ij})^{1/4}$ (Oliveira and Alencar, 1987). Since heifers were maintained in pasture after each infestation natural infestation could have occurred and a further data set using a transformed C_{ij} value, *i.e.* $CT_{ij} = \log_{10}(C_{ij} + 1)$ (Oliveira *et al.*,1989), was analyzed to confirm results of PR_{ij}. Data (PRT_{ij} and CT_{ij}) were subjected to analysis of variance (ANOVA) by the least squares method with a model that included the effects of genetic group (GG), animal within GG (error a, to test GG), infestation number (I), GG x I and the residual (error b). Results were also expressed as percentage tick-mortality (TM) by subtracting the percentage of return from 100.

Results and Discussion

A summary of the transformed percentage of return (PRT_{ij}) and the transformed tick count (CT_{ij}) ANOVA is presented in Table 1. All sources of variation included in the model significantly ($p < 0.01$) affected the traits studied and the model explained about 87% of the variation in the traits. The least squares means of the PRT_{ij} and CT_{ij} values (Table 2) and the untransformed number of ticks (C_{ij},

Table 1 - Summary of the analyses of variance of transformed percentage of return (PRT_{ij}) and transformed number of ticks counted (CT_{ij}).

Source of variation	Degrees of freedom	Mean Squares	
		PRT_{ij}^{a}	CT_{ij}^{b}
Genetic group (GG)	3	4.45	77.92
Animal/GG	62	0.23	4.01
Infestation	3	1.37	23.42
GG x infestation	9	0.21	2.97
Resídue	186	0.03	0.43
R^2 (%)		87	88

^{a} $(P_{ij})^{1/4}$; ^{b} $\log_{10}(C_{ij}+1)$.

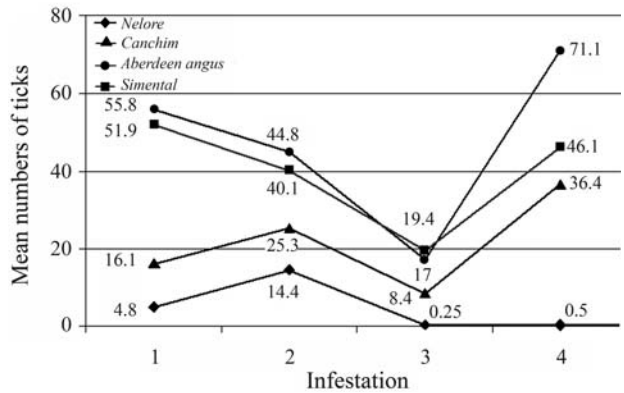

Figure 1 - Mean number of ticks according to infestation and genetic group.

Figure 1) are given according to genetic group and infestation. Angus x Nelore and Simmental x Nelore heifers were similar and presented higher PRT_{ij} values than Nelore and Canchim x Nelore heifers in all four infestations. However, although Nelore and Canchim x Nelore heifers showed similar PRT_{ij} values in the first two infestations the Nelore heifers showed lower PRT_{ij} values than Canchim x Nelore heifers in the last two infestations.

Despite the existence of genetic group x infestation interaction, in all four infestations, 1/2 European + 1/2 Nelore heifers showed higher PRT_{ij} values than heifers from the other groups. The estimated means were 0.35 ± 0.06 for Nelore, 0.54 ± 0.06 for Canchim x Nelore, 0.85 ± 0.06 for Simmental x Nelore and 0.89 ± 0.06 for Angus x Nelore (Table 2). Hence, Nelore heifers showed the lowest PRT_{ij} values (*i.e.* were the most tick-resistant), Canchim x Nelore heifers had intermediate PRT_{ij} values which were higher than Nelore but lower than Angus x Nelore and Simmental x Nelore, and Simmental x Nelore and Angus x Nelore heifers were similar and had the highest PRT_{ij} values. Our data shows that PRT_{ij} values increased with the proportion of *Bos taurus* genes in the heifers, and that even the Canchim x Nelore crossbred heifers, which were only 31.25% European because the Canchim breed is 5/8 Charolais (*B. taurus*) and 3/8 Zebu (*B. indicus*), were less resistant than the purebred Nelore heifers. These results support

Table 2 - Least squares means of the transformed percentage of return (PRT_{ij}) and transformed number of ticks counted (CT_{ij}), according to genetic group and infestation. The PRT_{ij} standard error (SE) was 0.04 for genetic group x infestation, 0.02 for infestation and 0.06 for genetic group. The CT_{ij} SE was 0.16 for genetic group x infestation, 0.08 for infestation and 0.25 for genetic group.

	PRT_{ij}				
	Infestation				
Cattle genetic group	1	2	3	4	Overall[1]
Nelore	0.41^{bB}	0.69^{aB}	0.09^{cC}	0.15^{cC}	0.35^{C} (0.10)
Canchim x Nelore	0.50^{bB}	0.71^{aB}	0.38^{cB}	0.59^{bB}	0.54^{B} (0.43)
Angus x Nelore	0.96^{aA}	0.94^{aA}	0.70^{bA}	0.96^{aA}	0.89^{A} (0.94)
Simmental x Nelore	0.94^{aA}	0.90^{aA}	0.68^{bA}	0.88^{aA}	0.85^{A} (0.79)
Overall	0.70^{b}	0.81^{a}	0.46^{d}	0.64^{c}	0.65
	CT_{ij}				
	Infestation				
Cattle genetic group	1	2	3	4	Overall[2]
Nelore	1.27^{bB}	2.44^{aB}	0.17^{cC}	0.31^{cC}	1.05^{C} (4.98)
Canchim x Nelore	1.71^{bB}	2.54^{aB}	1.26^{cB}	2.03^{bB}	1.89^{B} (21.54)
Angus x Nelore	3.66^{aA}	3.65^{aA}	2.48^{bA}	3.57^{aA}	3.34^{A} (47.16)
Simmental x Nelore	3.58^{aA}	3.48^{aA}	2.48^{cA}	3.30^{aA}	3.21^{A} (39.37)
Overall	2.56^{b}	3.03^{a}	1.60^{d}	2.30^{c}	2.37

[1,2]Values within parentheses are the estimated means of untransformed percentage return and untransformed number of ticks counted. Different uppercase letters in the same column indicate a significant difference for genetic group within infestation (t-test $p \leq 0.05$). Different lowercase letters in the same line indicate a significant difference for infestation within genetic group (t-test $p \leq 0.05$).

those of Oliveira and Alencar (1987), who reported higher PRT_{ij} values in Canchim than in Nelore cattle. Differences between genetic groups relative to PRT_{ij} values in artificial infestations have also been reported by Utech *et al.* (1978) who compared several genetic groups of cattle and found that *B. indicus* Brahman cattle were the most resistant, followed by *B. indicus x B. taurus* crossbreeds and then British *B. taurus* cattle. These authors also reported that among the *B. taurus* breeds studied Jersey heifers were more resistant than Guernsey, Australian Illawarra Shorthorn or Friesian heifers. In naturally infested animals, different degrees of infestation have also been reported in different genetic groups (Lemos *et al.*, 1985; Oliveira *et al.*, 1989; Oliveira and Alencar, 1990). Teodoro *et al.* (1994) observed a tendency for crossbred cows sired by Jersey bulls to show lower tick infestation than cows sired by Holstein and Brown Swiss bulls, although the differences were not statistically significant. Frisch (1997) classifies *B. indicus* African and Indian zebu cattle as highly resistant to cattle ticks, *B. taurus* Sanga cattle as a little less resistant, and British and continental *B. taurus* breeds as having low resistance. It has been suggested that the increased tick-resistance of *B. indicus* zebu cattle has evolved because cattle from tropical climates have always been in contact with ticks while *B. taurus* European cattle established contact with ticks only recently when *B. taurus* was introduced into the tropics (Andrade ABF, PhD Thesis, Faculdade de Ciências Agrárias e Veterinárias, UNESP, Jaboticabal, 2001).

Although the exact mechanisms of bovine tick-resistance are still not well known, Riek (1962) has classified them as innate resistance, present before the first infestation, and acquired resistance produced after the first infestation. O'Kelly and Spiers (1976) reported that when first exposed to ticks after birth crossbred zebu calves were more resistant than calves of European breeds, which suggests some degree of innate resistance. Some workers have suggested that the inoculation of foreign substances with the saliva of tick larvae produces irritation which results in self-cleaning (licking, abrading or rubbing) by the animals in an attempt to remove the ectoparasite (Kemp *et al.*, 1976; Koudstaal *et al.*, 1978). Riek (1962) and Willadsen *et al.* (1978) reported hypersensitive reactions in tick-resistant cattle that may result in ticks dropping off the cattle. Other mechanisms may also be related to tick-resistance, such as arteriovenous anastomosis in the dermal vasculature of *B. taurus* cattle as suggested by Schleger *et al.* (1981) and mast-cell counts in the skin of taurine and zebuine hosts as reported by Moraes *et al.* (1992).

In our study, the PRT_{ij} means for the different infestations were 0.70 ± 0.02 for the first infestation, 0.81 ± 0.02 for the second infestation, 0.46 ± 0.02 for the third infestation and 0.64 ± 0.02 for the fourth infestation, showing a significant reduction in the mean PRT_{ij} value for the third infestation. There was an increase in the PRT_{ij} and CT_{ij} values for Nelore and Canchim x Nelore heifers from the first to the second infestation, while Angus x Nelore and Simmental x Nelore heifers maintained high PRT_{ij} and CT_{ij} values in the first and second infestations (Table 2, Figure 1). The increase in the PRT_{ij} values from the first to the second infestation may, in part, have been due to the number of counts made, three counts having been made in the first infestation and five in the following infestations. In the third infestation, all genetic groups showed low PRT_{ij} values. In the fourth infestation, there was a significant increase in number of ticks on Canchim x Nelore, Angus x Nelore and Simmental x Nelore heifers, while Nelore heifers maintained a low level of infestation, suggesting that these heifers acquired a stable resistance after the third infestation. Another possibility is that because the heifers in our study were maintained on Brachiaria grass pasture throughout the experiment it is possible that natural infestations occurred and caused part of the variation between infestations. This natural infestation could have originated from preexisting larvae, or larvae remaining from previous artificial infestations, in the paddock the heifers were maintained and/or larvae from neighbor paddocks occupied by other groups of animals. It is also possible that a more pronounced natural infestation occurred between the third and the fourth infestations, when climatic conditions were more favorable to ticks. Tick development between the 2nd and the 3rd counts might have been affected by variations in climatic variables (Figure 2) and this could be the reason for the decrease in number of ticks in the 3rd infestation.

In regard to acquired resistance, Riek (1962) studied *B. taurus*, *B. indicus* and their crosses and found that although acquired resistance was least apparent in purebred *B. taurus* there was considerable variation in the degree of resistance between individual cattle within breed groups. Wagland (1975) compared *B. indicus* Brahman and *B. taurus* Shorthorn cattle during four successive infestations with *B. microplus* larvae and obtained a similar number of engorged females after the first infestation in both breeds, however, in the fourth infestation Brahman heifers had significantly less engorged females than Shorthorn heifers. In a subsequent study, Wagland (1978) found that Brahman

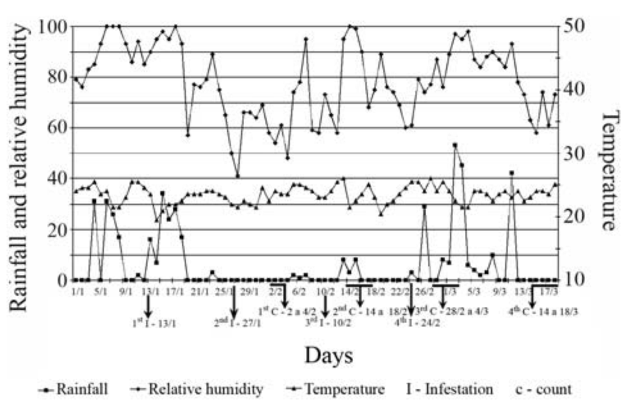

Figure 2 - Relative humidity, temperature and rainfall for January to March 2005.

Table 3 - Cattle genetic group and number (N) and percentage (%) of heifers by tick-mortality class.

	Tick-mortality class[1]					
	> 99.0%		> 98.0% and ≤ 99.0%		> 97.0% and ≤ 98.0%	
Cattle genetic group	N.	%	N.	%	N.	%
Nelore	16	100.00	-	-	-	-
Canchim x Nelore	15	83.33	3	16.67	-	-
Angus x Nelore	11	68.75	3	18.75	2	12.50
Simmental x Nelore	10	62.50	5	31.25	1	6.25

[1]Tick-mortality obtained by subtracting the mean percentage of return (PRT_{ij}) of the four infestations from 100.

heifers developed measurable degrees of tick-resistance during the first three days of infestation while Shorthorn heifers develop tick-resistance only after 20 days, indicating that as well as the innate resistance component, which varies among breeds, there is a significant acquired resistance component. Barriga *et al.* (1993) studied *B. microplus* infestations of *B. taurus* Hereford cattle but found no relationship between natural resistance and the ability to develop acquired resistance. In Barriga's study, although the cattle were homogeneous in breed, sex, age and maintenance conditions during the first infestation, when the cattle had no previous contact with ticks, distinct resistance groups were established for tick functions such as duration of feeding and the start of egg-laying and hatching. This suggests that cattle belonging to one initial group segregated into distinct groups, supporting an heterogeneous acquired immunological response. High degrees of tick-resistance have been associated with zebu cattle and their crossbreeds, probably due to the adaptive ability of such cattle, which, among other aspects, is expressed by coat characteristics, such as the short-hair and smooth-hair traits.

The intraclass correlation (IC), based on the heifer nested within genetic group and the residual components of variance, was used as a measure of the repeatability of the PRT_{ij} values and was estimated to be IC = 0.65 ± 0.05. This value indicates the correlation between the PRT_{ij} values of any two infestations and that the first infestation would be 65% as accurate in estimating the PRT_{ij} values in the second infestation. We found that the average of all four infestations would be 88% as accurate in estimating the PRT_{ij} values in a fifth infestation, representing an increase of 35% in the accuracy relative to only one measurement. This value of repeatability and the variations observed in the PRT_{ij} values for the four infestations suggest that more than one infestation should be done when evaluating the resistance of heifers to cattle ticks. The repeatability obtained in this study is higher than the value of 0.29 reported by Fraga *et al.* (2003) for naturally infested Caracu cattle. This was to be expected because the environmental conditions in our study were more controlled than in natural infestations with long periods between counts during which there can be physiological changes in cattle, climate and pasture.

Utech *et al.* (1978) divided tick-resistance in cattle into the following tick-mortality (TM) classes: > 98% TM, highly tick-resistant cattle; 95.1% to 98% TM, moderately tick-resistant; 90% to 95% TM, low tick-resistance; and < 90% TM, very low tick-resistance. The observed TM based on the mean PRT_{ij} values of the four infestations (Table 3) shows that a TM above 99% occurred in the following percentage of heifers: 100% for Nelore; 83.33% for Canchim x Nelore; 68.75% for Angus x Nelore; and 62.5% for Simmental x Nelore. Furthermore, about 16.67% of the Canchim x Nelore heifers were included in the > 98% to ≤ 99% TM group, which also included 18.75% of the Angus x Nelore and 31.25% of the Simmental x Nelore heifers. Based on the classification of Utech *et al.* (1978) all (100%) of the Nelore heifers and the Canchim x Nelore heifers would be considered highly tick-resistant while only 87.5% of the Angus x Nelore heifers and 93.75% of the Simmental x Nelore heifers would be considered highly tick-resistant, the remaining percentage of Angus x Nelore heifers (12.5%) and Simmental x Nelore heifers (6.25%) being moderately tick-resistant. Since Canchim x Nelore heifers have, on average, a higher proportion of *B. indicus* zebu genes the higher percentage of high tick-resistance shown by these heifers as compared to Angus x Nelore and Simmental x Nelore heifers, with a relatively low percentage of *B. indicus* genes, was to be expected.

Considering all four infestations, Angus x Nelore and Simmental x Nelore heifers showed a higher percentage of tick return than Nelore heifers, while Canchim x Nelore heifers reached an intermediate degree, suggesting higher resistance to cattle tick in Nelore heifers, intermediate resistance in Canchim x Nelore heifers, and lower resistance in Angus x Nelore and Simmental x Nelore heifers. Nevertheless, most of the Angus x Nelore and Simmental x Nelore heifers can also be considered highly resistant.

Acknowledgments

This study was *supported by* the Brazilian *Federal* Research Council (Conselho Nacional de Desenvolvimento Científico e Tecnológico – CNPq)

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Associate Editor: Fábio de Melo Sene