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Field study on nematode resistance in Nelore-breed cattle

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Abstract

The present study evaluated Nelore cattle with different degrees of resistance to natural infections by gastrointestinal nematodes. One hundred weaned male cattle, 11–12 months of age, were kept on the same pasture and evaluated from October 2003 to February 2004. Faecal and blood samples were collected for parasitological, haematological and immunological tests. In February 2004, the 10 most resistant and the 10 most susceptible animals were selected based on individual means of nematode faecal egg counts (FEC). Such animals were slaughtered for worm burden determination and nematode species identification. The repeatability estimates for FEC (±S.D.), log-transformed FEC and packed-cell volume (PCV) in all animals were 0.3 (±0.05), 0.26 (±0.04) and 0.42 (±0.05), respectively. The resistant group showed lower FEC and worm burdens than the susceptible group ($P < 0.05$). There were no significant differences between groups regarding mean body weight, weight gain, PCV and total serum protein values ($P > 0.05$). The resistant group showed higher total serum IgE levels ($P < 0.05$) and higher mean eosinophil blood counts. However, the latter was statistically significant only 42 days after the beginning of the study. Nematodes *Cooperia punctata* and *Haemonchus placei* were predominant and the correlation between *Cooperia* and *Haemonchus* burdens was 0.64 ($P < 0.05$), which indicated that animals presenting increased numbers of one of those genera probably had increased numbers of the other. The current study provides further evidence of IgE active role in nematode immunity and suggests that total serum IgE level might serve as an additional marker to select Nelore cattle that are responsive to *H. placei* and *C. punctata* infections.

Keywords: Cattle; *Cooperia*; *Haemonchus*; *Oesophagostomum*; Nelore breed; IgE; Resistance

1. Introduction

Most beef cattle raised in Brazil belong to Nelore breed (*Bos indicus*). These Zebu cattle were brought to Brazil from Asia in the 18th century (Santiago, 1986) and showed excellent adaptation to the environmental and husbandry conditions found in most Brazilian states. In addition, this breed of cattle is highly resistant to tick (*Boophilus microplus*) infestation.

Parasite control mainly relies on anthelmintic treatment but the increasing demand for chemical-free animal products and the development of anthelmintic resistance to several chemical groups in parasites require new control strategies like the selection of resistant hosts.

The most used method to identify resistant hosts is still faecal egg counts (FEC), which is directly related to

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worm number and parasite fecundity (Sonstegard and Gasbarre, 2001). Studies using cattle in tropical areas have also indicated a significant correlation between FEC and total worm burdens (Bryan and Kerr, 1989).

Several studies in sheep have shown that immunoglobulin (Ig) E and eosinophil levels are also associated with parasite resistance. IgE production is generally considered an important factor in the host response to helminth infection (Miller, 1996). Bendixsen et al. (2004) showed that antigen-specific levels of IgE were significantly higher in genetically resistant lambs, compared with genetically susceptible lambs, after infection by *Trichostrongylus colubriformis*. Pettit et al. (2005) selected 10 responsive and 10 non-responsive lambs among a Scottish Blackface flock infected with *Teladorsagia circumcincta* and found a larger number of circulating IgE-bearing cells in the blood of responsive lambs than in that of non-responsive lambs.

Some studies in cattle have measured serum IgE levels following natural infections (Baker and Gershwin, 1992; Miller et al., 1996). The results obtained were not consistent but, in general, serum IgE levels increased after mild helminth infections. In a study about *Dictyocaulus viviparous* infection in calves, IgE levels were higher in animals previously infected than in non-infected controls from day 14 onwards. Among the lungworm-infected group, there was a significant correlation between total serum IgE levels and protection from 2 to 4 weeks after infection (Kooyman et al., 2002).

The present study aimed at identifying resistant and susceptible young Nelore cattle exposed to natural infection by gastrointestinal nematodes. Resistant and susceptible groups were compared regarding worm burden, PCV, weight gain, number of blood eosinophils, total plasma protein and IgE levels in the animals' sera.

2. Material and methods

2.1. Animals and experimental design

One hundred Nelore young bulls were evaluated. They were raised in a farm located at Londrina, Paraná State, south of Brazil (longitude 23°18′37″S, latitude 51°09′46″W, average annual temperature 21.3 °C and rainfall in all seasons). Animals were born in November and December 2002. They were kept on 79 ha (divided into four paddocks) of *Panicum maximum* pasture, which was naturally contaminated by nematode larvae. The bulls were subjected to the farm's usual sanitary and nutritional management. They were treated with doramectin (200 μg/kg; Dectomax®, Pfizer) soon after birth to prevent myiases and were vaccinated against clostridiosis twice (Fortress®, Pfizer; in March 2003 and June 2003). At weaning, around 6 or 7 months of age, they received anthelminthic treatment with ivermectin (Ivomec Gold®, Merial) and were vaccinated against foot and mouth disease.

At the beginning of the experimental observations (October 31, 2003), animals were between 11 and 12 months of age. All bulls were kept on the same pasture and did not receive any additional anthelminthic treatment until the end of the study (February 19, 2004).

2.2. Measurements

Each animal was weighed and faecal and blood samples collected fortnightly. Based on the mean FEC obtained from October 31, 2003 to January 26, 2004, the 10 most resistant (i.e. those presenting the lowest mean FEC) and the 10 most susceptible animals (i.e. those presenting the highest mean FEC) were selected for worm burden determination and designated as resistant group and susceptible group, respectively. The selected animals were planned to be sacrificed immediately after the determination of FEC in January 26; however, problems in the slaughterhouse led to the postponement of the sacrifice until February 20, 2004.

2.2.1. Parasitology

Faecal samples were collected directly from the rectum for FEC determination, which was carried out using a modified McMaster technique (Ueno and Gonçalves, 1998). Infective larvae were identified according to descriptions of Keith (1953).

At necropsy, the abomasum and the small and large intestines were removed, opened, and their contents placed in a graduated bucket. A 5% aliquot was preserved in 5% formalin for subsequent enumeration and identification of nematodes into genus based on their morphology (Amarante et al., 1997; Ueno and Gonçalves, 1998). All adult males present in the aliquots were identified to species based on the morphology of their spicules.

2.2.2. Haematology

Blood samples with and without anticoagulant (EDTA) were collected through jugular vein puncture fortnightly from November 17, 2003 to January 26, 2004. Packed cell volume (PCV) was determined using the microhematocrit method and total serum protein levels were assessed using a refractometer (Refractometer SPR-N, Atago). Serum samples were stored at −20 °C

until use for immunoglobulin measurements. Eosinophil counts from peripheral blood were carried out in a Newbauer's chamber after Carpentier's solution staining (Dawkins et al., 1989) and expressed as number of cells per μl blood.

2.2.3. Serology—enzyme-linked immunosorbent assay (ELISA) for IgE antibody

Total IgE levels in the animals' sera were measured using sandwich ELISA as previously described (Kooyman et al., 2002).

2.3. Statistical analysis

Repeatability for FEC and PCV was estimated over time (Giannoni and Giannoni, 1987). Animals that had missing FEC or PCV data were excluded from the analysis. The total number of animals evaluated for repeatability was 91 (FEC) and 95 (PCV).

Significant differences between the resistant and the susceptible group regarding FEC, PCV, body weight, worm burden, number of blood eosinophils, total serum protein and IgE levels were assessed through analysis of variance using the General Linear Model (GLM) of the Statistical Analysis Systems Institute (SAS, 1989). Faecal egg counts, worm burden, eosinophil counts and total IgE levels were transformed using $\log_{10}(x + 1)$ to stabilise variance before the analysis. Relationships among the transformed data were established using Pearson's correlation.

Results were expressed as arithmetic means (±standard deviation) of non-transformed data.

3. Results

3.1. Parasitology

The repeatability estimates for FEC (±S.D.) and log-transformed FEC were 0.3 (±0.05) and 0.26 (±0.04), respectively. The frequency distribution of individual mean FEC of 100 animals is presented in Fig. 1. Throughout the study, most of the animals (55%) showed individual mean FEC lower than 200 eggs/g (EPG), whereas only 14% animals had mean FEC higher than 500 EPG.

Animals of the resistant group showed lower FEC than those of the susceptible group ($P < 0.05$) (Fig. 2). The resistant group showed FEC mean values lower than 50 throughout the experimental period, except on the slaughter day when up to 195 EPG were observed. The susceptible group presented means FEC constantly

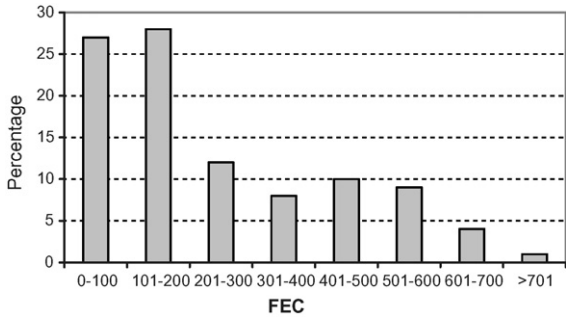

Fig. 1. The percent distribution of individual mean of faecal egg counts (FEC) of 100 Nelore young cattle. Data obtained fortnightly from October 31, 2003 to January 26, 2004.

higher than 400 EPG and a peak was noticed on the 14th day.

In most of the experimental weeks, *Cooperia* spp. was the predominant genus found in composite cultures followed by *Haemonchus* spp. and *Oesophagostomum* spp. Larvae from the latter were identified in the cultures from the 14th day onwards showing higher percentages in the last weeks of the study.

The resistant group showed lower mean *Haemonchus* spp. and *Cooperia* spp. burdens than the susceptible group (Table 1). The estimated mean numbers of *Haemonchus placei* and *Haemonchus similis* were, respectively, 98.8 and 41.2 in the resistant group and 1092.4 and 127.6 in the susceptible group. In average, 1493.3 *Cooperia punctata* and 6.7 *Cooperia pectinata* specimens were found in the resistant group and 4778.5 *C. punctata*, 19.7 *Cooperia spatulata* and 6.8 *C. pectinata* in the susceptible group.

Oesophagostomum radiatum was the main parasite present in the large intestine of both groups at similar

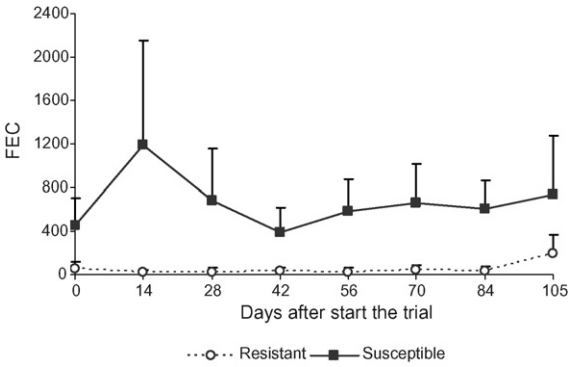

Fig. 2. Means of faecal egg counts (FEC) of the resistant ($n = 10$) and susceptible ($n = 10$) groups of Nelore cattle, naturally infected with gastrointestinal nematodes. Data obtained from October 31, 2003 to February 20, 2004, day of the sacrifice of the animals. Bars are standard deviation. There was a significant ($P < 0.05$) difference between the groups on all data.

Table 1
Mean number of nematodes in the resistant ($n = 10$) and susceptible ($n = 10$) groups of Nelore young bulls naturally infected by gastrointestinal nematodes

Nematodes	Resistant	Susceptible	Significance
Haemonchus spp.			
Adults	140 (0–285)	1220 (0–2679)	**
Immatures	16 (0–80)	52 (0–200)	
Total burden—abomasum	156 (0–380)	1272 (80–3400)	**
Cooperia spp.			
Adults	1500 (0–7620)	4805 (20–12,260)	*
Immatures	0	2 (0–20)	
Total burden—small intestine	1500 (0–7620)	4807 (20–12,260)	*
Oesophagostomum spp.			
Adults	100 (0–260)	174 (0–760)	
Trichuris spp.			
Adults	20 (0–40)	12 (0–20)	
Immatures	2 (0–20)	10 (0–60)	
Total burden—large intestine	122 (0–280)	196 (60–760)	
Total worm burden	1778 (40–7800)	6275 (160–13,900)	*

Minimum and maximum values are in parenthesis. Significant differences between worm burdens of different groups are indicated.
* $P < 0.05$.
** $P < 0.01$.

quantities: in average 100 in the resistant group and 174 in the susceptible group. Small numbers of *Trichuris globulosa* and *Trichuris discolor* were recorded without any significant difference between groups.

3.2. Haematology, serology and body weight

Nematode infection was asymptomatic even in susceptible animals. No differences between groups were noticed regarding mean PCV and total serum protein values (Fig. 3). The repeatability estimate for PCV was 0.42 (±0.05). Body weight increased throughout the study (Fig. 4). No significant differences between groups were observed regarding mean body weight and weight gain.

The resistant group presented higher blood eosinophil counts (2294 ± 945) on the 42nd day, compared with the susceptible group (930 ± 945) ($P < 0.05$) (Fig. 5). In all experimental weeks, the resistant group had significantly higher total serum IgE levels compared with the susceptible group (Fig. 6).

3.3. Correlations

The correlation coefficients regarding FEC (log-transformed), weight gain and PCV which were calculated using data from 100 animals at every sampling time were, in general, close to zero. A significant correlation coefficient was only found between FEC and

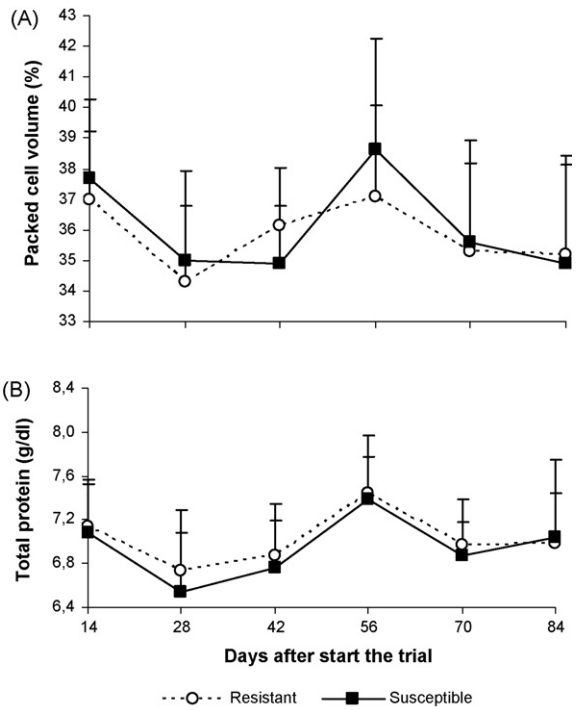

Fig. 3. Arithmetic means of (A) packed cell volume and (B) total plasma protein of the resistant ($n = 10$) and susceptible ($n = 10$) groups of young Nelore bulls naturally infected with gastrointestinal nematodes. Bars are standard deviation. No significant ($P < 0.05$) difference was found between the groups.

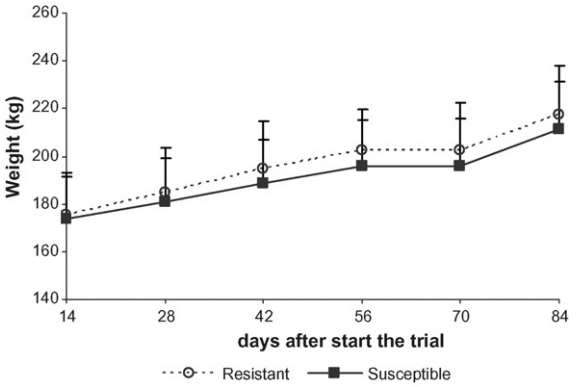

Fig. 4. Arithmetic means of body weight (kg) of the resistant ($n = 10$) and susceptible ($n = 10$) groups of Nelore cattle, naturally infected with gastrointestinal nematodes. Bars are standard deviation. No significant ($P < 0.05$) difference was found between the groups.

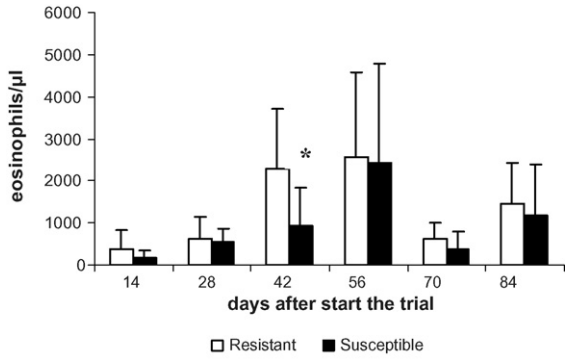

Fig. 5. Means of blood eosinophils of the resistant ($n = 10$) and susceptible ($n = 10$) groups of Nelore cattle naturally infected with gastrointestinal nematodes. Bars are standard deviation. Data on which a significant ($P < 0.05$) difference was found between the groups are indicated with an asterisk (*).

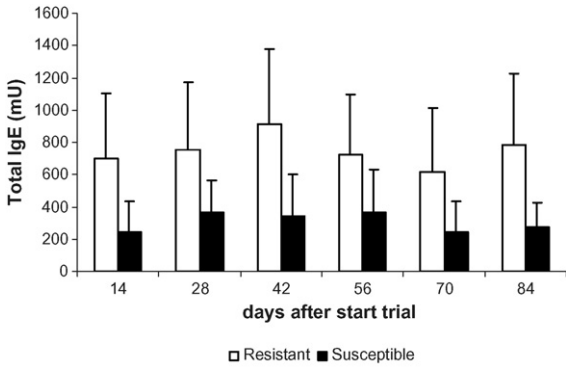

Fig. 6. Means of total IgE concentration in sera of the resistant ($n = 10$) and susceptible ($n = 10$) groups of Nelore cattle naturally infected with gastrointestinal nematodes. Bars are standard deviation. There was a significant difference between the groups on all data ($P < 0.05$).

PCV on the 14th day ($r = 0.21$; $P < 0.05$). Analysing the 20 selected animals, correlation coefficients between the worm burdens of the two major parasites (*Haemonchus* and *Cooperia*, $r = 0.64$; $P < 0.05$) and between FEC and total worm burdens ($r = 0.47$; $P < 0.05$) were positive, whereas the correlation between IgE levels and total worm burdens was negative ($r = -0.42$; $P = 0.06$).

4. Discussion

Parasite burdens have generally been over-dispersed, and negative binomial distribution has provided a good empirical description of dispersed distributions: most hosts carry few parasites, whereas a few heavily infected hosts harbour a large proportion of the total parasite population (Barger, 1989). Such over-dispersed pattern was clearly observed in the present study corroborating a previous experiment using Nelore young cattle (Nicolau et al., 2002).

No clinical signs of gastrointestinal infections or accentuated weight loss were noticed, and there was no association among FEC, PCV and weight gain. Similarly, Nicolau et al. (2002) did not notice any association among those variables in naturally infected Nelore cattle. In another study carried out in Australia using crossbred Zebu animals, there was a low correlation between resistance to parasites and growth (Mackinnon et al., 1991).

In the current study, the resistant group showed FEC mean values lower than 50 throughout the experimental period, except on the slaughter day when FEC reached 195 EPG. As the animals were kept without solid food for 24 h before slaughter, eggs were probable more concentrated in a smaller amount of intestinal contents.

The repeatability value obtained for FEC (0.3) was similar to the values found by Stear et al. (2000) in sheep and by Mackinnon et al. (1991) in crossbred Zebu and Nicolau et al. (2002) in Nelore cattle naturally infected with nematodes. Repeatability for PCV (0.42) could be considered moderate and thus could be used together with the FEC to monitor nematode infections. This value was similar to the results obtained by Bekele et al. (1992) in sheep.

C. punctata and *H. placei* were the predominant parasites found in cattle, which agrees with previous studies carried out in Brazil (Guimarães et al., 1990; Lima, 1998; Nicolau et al., 2002) and in other tropical countries (Durie, 1962; Bryan and Kerr, 1989). Correlation coefficients between those parasites were positive, demonstrating that animals with increased numbers of one genus probably had increased numbers of the other. Stear et al. (1998) and Amarante et al.

(2004) found similar results in naturally infected sheep. Such association indicates that the selection for resistance to one of the species will consequently improve the resistance to the other. With regard to the association involving other species found, the results were not conclusive because the numbers of such species were relatively low.

Results indicated that even breeds well-adapted to the tropics include individuals susceptible to nematode infections, presenting high worm burdens that result in increased pasture contamination and, consequently, in a higher degree of herd exposure to the parasites. Selecting gastrointestinal parasite-resistant animals may be an efficacious alternative for the prophylaxis of gastrointestinal nematode infections, minimizing the need for anthelminthic treatments, thus reducing the rate of development of anthelmintic resistance in the parasites. Such problem has been reported in countries that use pasture systems to raise beef cattle, including New Zealand (Vermunt et al., 1995) and Argentina (Fiel et al., 2001). A recent Brazilian study in cattle indicated the presence of *Cooperia* spp. and *Haemonchus* spp. resistant to several anthelmintics, particularly ivermectin (Soutello, 2005).

Not only FEC but also total serum IgE level was associated with the resistance degree, corroborating the results obtained by Kooyman et al. (1997) and Pettit et al. (2005) in sheep. Therefore, the present study provides further evidence of a role of IgE in nematode immunity and suggests that total serum IgE level might serve as an additional marker for selecting Nelore cattle that are responsive to *H. placei* and *C. punctata* infections.

Acknowledgments

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