



## ANIMAL SCIENCE

# Phenotypical categorization of indigenous ewes as resistant, resilient or susceptible to *Haemonchus contortus*

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**Abstract:** The economic losses caused by parasite infections, aggravated by resistance to anthelmintics, have generated demand for alternatives involving non-chemical control, such as the selection of resistant animals. The objective of this study was to identify which characteristics best describe animals that are resistant, resilient or susceptible to *Haemonchus contortus* and estimate the percent number in each category. Sixty-one Morada Nova ewes were evaluated in an extensive system. The performance variables (weight, body condition score), hematological variables (packed cell volume, hemoglobin, white blood cells, segmented neutrophils, lymphocytes, eosinophils, monocytes) and fecal egg counts were measured individually every 14 days during 6 months. The variables were transformed, and analysis of variance was carried out, with construction of a correlation network. Characteristics linked to parasite infection showed variations among the categories, which helped to identify sheep resistant, resilient or susceptible to *H. contortus*. Based on the analyses performed, 88.3% of the animals were resistant or resilient and only 11.7% were susceptible. Presence of Trichostrongylidae eggs, body condition score, packed cell volume (PCV) and eosinophil counts were found to be good indicators of naturally infected ewes, and there were significant differences between the categories and correlations between the traits.

**Key words:** Nematodes, hematology, *Ovis aries*, network correlation.

## INTRODUCTION

The maintenance of sheep in pastures is common, resulting in their constant exposure to the challenge of infection by gastrointestinal parasites, the main factor responsible for production losses. The most economically important gastrointestinal parasite of sheep in tropical and subtropical regions is *Haemonchus contortus*. Infection by this parasite can cause decreased performance and even death of animals (MacKinnon et al. 2015). Treatment with anthelmintics is the most common control strategy and is considered one of the largest

production costs in many countries (Vlassoff & Mckenna 2010).

In addition to the high expense for anthelmintics, the desire to eliminate parasites leads farmers to increase the number of deworming's, using different chemicals found in the market. This action has a negative impact on the production system, contributing to the selection of drug-resistant parasite populations (Manikkavasagan et al. 2015, Brom et al. 2013). Due to this, many studies have been conducted to find alternatives that can reduce the use of

anthelmintics and also reduce the losses caused by parasitic infections (Valilou et al. 2015).

The selection of animals that show inherent resistance, combined with other control strategies, is one of the most promising approaches for parasite control (Brown & Fogarty 2017). Resistance to gastrointestinal parasites is not an absolute trait, nor is it limited only to certain breeds, due to the variability between and within herds. However, some breeds may be more adaptable and have better responses to infection challenge (MacKinnon et al. 2015).

The Morada Nova is one of the main indigenous hair sheep breeds of Northeast Brazil (Muniz et al. 2016), although this is little known. McManus et al. (2009) highlighted its potential for use in intensive meat production, taking into account the resistance to worms and good reproductive performance, producing heavier lambs at birth and at weaning in industrial crosses, as also observed by Issakowicz et al. (2016).

The host resistance is measurable through performance after infection. The performance of an animal can be evaluated through productive characteristics, the clinical condition observed, immunological responses and variables directly related to parasite presence, such as fecal egg count (Ferreira et al. 2017).

Resolution of whether there are unfavorable relationships between fecal egg count and productive traits, and under what conditions they may exist, is one of the most important aspects facing sheep genetic research (Valilou et al. 2015). Thorough knowledge of the genetic and immunological mechanisms of the relationship can suggest methods by which both production and parasite resistance can be improved.

Based on knowledge of phenotypic markers used to identify physiological changes due to parasitic infection by *Haemonchus contortus*, this study estimated the percentage of resistant,

resilient or susceptible animals, and identified the differences in phenotypic traits that best describe each category.

## MATERIALS AND METHODS

This animal experiment was performed with the approval of the Committee on Ethical Use of Universidade Estadual do Norte Fluminense Darcy Ribeiro (CEUA-UENF) and according to the standards of the Sociedade Brasileira De Ciência Em Animais De Laboratório/Comissão Nacional de Bem-Estar Animal (SBCAL/COBEA), according to protocol no. 317.

### Locale, animals and management

This study was conducted at the Research Center on Diversified Animal Husbandry, Animal Science Institute, São Paulo Agency of Agribusiness and Technology (APTA), part of the São Paulo State Agriculture and Supply Secretariat, located in Nova Odessa, São Paulo (22°42' S and 47°18' W).

According to the Köppen classification, the climate in the region is C<sub>Wa</sub>, characterized as tropical with relatively rainy and hot summers and dry winters. The average annual rainfall is 1,317 mm and the temperature is between 10 °C and 35 °C, with an average of 26 °C. The average annual relative humidity is approximately 75%.

A total of 61 adult ewes of the Morada Nova breed, with an average age of 3 years and 30 (±4.9) kg average weight, were used in this study. The animals were naturally infected and managed under rotational grazing in an area of 6 hectares with an average capacity of 25 ewes per hectare. The pastures were formed by *Megathyrus maximus* Jacq. Cv. Aruana, and the animals had access to water and mineral mixture *ad libitum*.

### Collecting samples and evaluating animals

Blood and stool samples were collected every 14 days, at which time the sheep were also weighed and evaluated for body condition. The data were collected continuously for six months, including the dry (June-August) and rainy (September-November) seasons.

The body condition score was determined by palpation of the lower back, with assignment of values on a scale from 1 to 5 (Sanudo & Sierra 1986), also using half-point scoring according to Pugh (2005). Parasitological and hematological analyses were performed at the Animal Science Institute of Nova Odessa/APTA.

### Parasitological examinations

Stool samples were collected directly from the rectum for fecal egg counts (FEC) were conducted according to the modified technique of Gordon & Whitlock (1939), and infective larvae were identified to the genus level by fecal culture (Roberts & O'Sullivan 1950).

### Hematological analysis

Blood samples were collected by venipuncture of the jugular vein of all animals in Vacutainer tubes (5 ml) containing ethylenediaminetetraacetic acid (EDTA). The packed cell volume (PCV), hemoglobin (HGB), white blood cells (WBC) and red blood cells (RBC) were measured with an electronic hematological analyzer (Sysmex pochH-100iV Diff, Europe). Data were collected to identify blood and differential counts of segmented neutrophils (Seg Neut), lymphocytes (Lymph), eosinophils (Eosin), monocytes (Mono), basophils (Baso) and rods (Bast) (Schalm & Carroll 1986). There were no rods, so this parameter was removed from the statistical analysis.

### Animal classification

To better observe the phenotypic differences related to responses to gastrointestinal parasites, the animals were separated into groups based on the results of the tests performed. The criteria for classification as resistant, resilient or susceptible were based on fecal egg count (FEC), considering Trichostrongylidae, along with PCV and weight.

These characteristics were initially chosen to identify the animals that could remain free of infection through the FEC result. PCV was determined to check the physiological state of the animals, since most pathogenic parasites are hematophagous and weight indicates whether or not an animal is parasitized. The FEC and PCV values of each category were determined according to the minimum and maximum values observed in the herd and the repeatability of the animals in remaining free of infection.

The categories were: 1-Resistant animals, showing low FEC (0-500), PCV values above 25%, and little or no weight variation; 2- Resilient animals, with FEC count of 0 to 3000 eggs, constant or recurrent infection, high PCV values (above 25%), and little or no weight variation; and 3- Susceptible animals, with constant infection, FEC values between 100 and 8000, low PCV values (below 25%) and weight oscillations.

### Statistical analysis

The assumption of normal distribution of all variables was checked (PROC UNIVARIATE, SAS, 2016). The variables that were not normally distributed (white blood cells, segmented neutrophils, lymphocytes, eosinophils, monocytes, Trichostrongylidae, *Strongyloides* spp., *Moniezia* spp. and *Eimeria* spp.) were transformed ( $\log^{-10}+1$ ). The means presented have original scale.

For all variables, analysis of variance was carried out with PROC GLM (SAS 2016),

considering repeated measures. The model considered the effect of weight as a covariate in addition to the fixed effects of categories, season and their interaction. The means were compared by the Tukey test at 5% probability. Pearson correlation coefficients were calculated for the characteristics of weight, body condition score, fecal egg count, red blood cells, hemoglobin, packed cell volume, white blood cells, segmental neutrophils, lymphocytes, eosinophils, and lymphocytes.

For the construction of the networks, we used Pearson correlations between the phenotypic means of pairs of characteristics. Correlation networks offer another way to investigate pairwise correlations and consist of a set of nodes connected by a system of edges. The thickness of edges was determined by applying a cutoff value equal to 0.3, meaning that only  $|r_{ij}| \geq 0.3$  had edges highlighted, and the proximity between nodes (traits) was proportional to the correlation value between those nodes. Positive correlations were colored dark green, whereas the negative ones were depicted in red. The analyses were performed using the software R version 3.1.2 (R Core Team 2015). The correlation network procedure was performed using the package “qgraph” (Epskamp et al. 2012).

## RESULTS

The parasitological evaluation found different types of eggs (Trichostrongylidae, *Strongyloides* spp., *Moniezia* spp.) and oocysts (*Eimeria* spp.). According to the coproculture results, the most prevalent larval genus found was *Haemonchus* spp. (92.3%), followed by the genera *Trichostrongylus* spp. (3.4%), *Cooperia* spp. (3.1%) and *Oesophagostomum* spp. (1.2%).

For the separation of the categories, only the variables Trichostrongylidae egg count, PCV

and weight were considered. Maximum values up to 8,000 FEC were observed, while some animals maintained very low or zero FEC during all evaluations (Figure 1 and Figure 2).

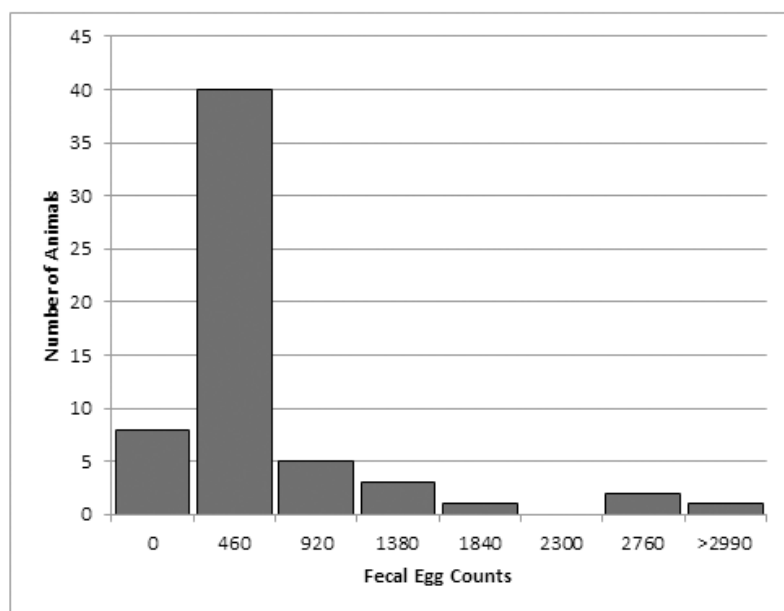
There were no statistical differences ( $p > 0.05$ ) for the FEC and PCV characteristics due to the season of the year, so the analyses were not separated by dry and rainy season. Figure 3 shows the difference of infection degree by Trichostrongylidae among the categories during the dry and rainy seasons.

Averages for weight (Table I) were statistically similar ( $p > 0.05$ ) between resistant ( $31.5 \pm 3.6$ kg) and resilient ( $31.5 \pm 5.5$ kg) animals, but different for the susceptible category ( $p > 0.05$ ), with lower weight values ( $26.5 \pm 2.7$ kg). For BCS, variations were observed, and the categories were different among them:  $2.19 \pm 0.6$ ,  $1.9 \pm 0.7$  and  $1.3 \pm 0.5$  ( $p > 0.05$ ) for resistant, resilient and susceptible, respectively.

The overall mean FEC values for Trichostrongylidae of the three categories were statistically different ( $p < 0.05$ ) (Table I). Figure 1 (a) shows the mean FEC values for Trichostrongylidae throughout the experiment according to the categories. There were differences ( $p < 0.05$ ) between the average of *Strongyloides* spp. for the different categories: the susceptible category showed higher averages while resistant and resilient showed similar averages with lower values. No differences ( $p > 0.05$ ) were observed for the averages of *Moniezia* spp. and *Eimeria* spp. among the categories.

The hematological variables were different ( $p < 0.05$ ) among the categories (Table II).

The averages of PCV, HGB, and RBC were similar for the resistant and resilient categories ( $p > 0.05$ ). The susceptible category showed the lowest values of PCV, HGB, and RBC, and differed statistically from the other categories ( $p < 0.05$ ). The variations of the PVC values during the



**Figure 1. Frequency distribution of the 60 Morada Nova ewes in relation to fecal egg count (FEC). From this, 45% of the animals were categorized as resistant, 43.3% resilient and 11.7% susceptible (Figure 2).**

experiment according to the categories can be seen in Figure 2b.

There were no significant differences between the resistant and resilient categories for WBC, and the susceptible category showed the lowest averages for WBC ( $4.68 \pm 1.0\%$ ).

Seg. Neut. showed difference just for the resistant category, with the lowest relative value ( $42.8 \pm 13.0\%$ ), while the resilient and susceptible categories were similar ( $p > 0.05$ ). There were no significant differences ( $p > 0.05$ ) among categories for Lymph and Mono. Eosin showed statistical difference among the categories ( $p < 0.05$ ).

Figures 4 (a for resistant, b for resilient and c for susceptible) show the constructed network with pairwise phenotypic correlations between characteristics of animals naturally infected by gastrointestinal parasites considering resistant, resilient and susceptible categories. Red lines represent negative correlations and green ones denote positive correlations, while the width of the line is proportional to the strength of the correlation. Only the significant characteristics are shown in the correlation network; the

non-significant correlations were excluded from the network for better observation.

### Resistant

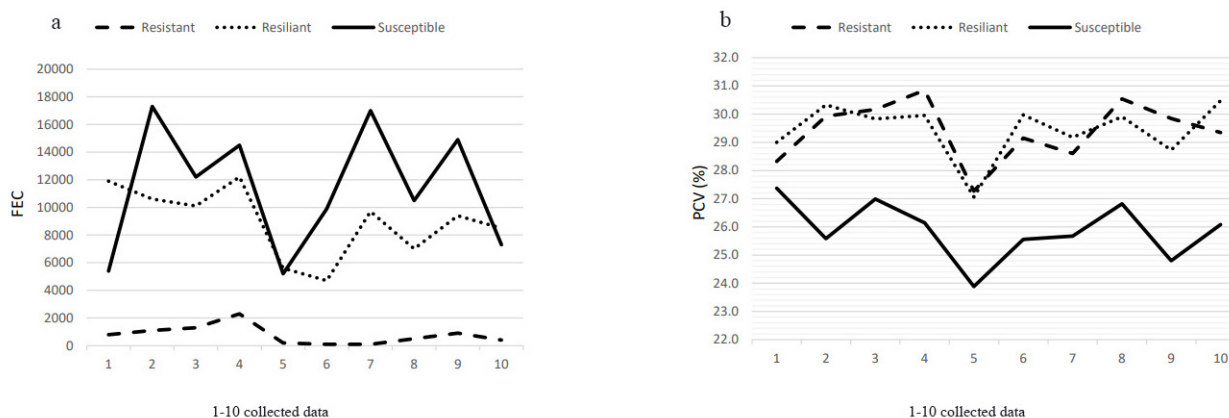
For the resistant category, the correlation between weight and BCS was positive and had medium magnitude,  $r=0.46$  ( $p < 0.01$ ).

The correlations between BCS and PCV and BCS and HGB were positive ( $p < 0.01$ ) with a coefficient of medium magnitude ( $r=0.24$ ;  $r=0.25$  respectively), while the correlation between weight and PCV was low and not significant ( $p > 0.05$ ).

The correlations between PCV and HGB, in all three categories, were positive and the coefficients were high, as expected ( $r=0.89$  resistant,  $r=0.88$  resilient and  $r=0.97$  susceptible).

For the resistant category, the correlation of PCV with Trichostrongylidae was low and not significant ( $r=0.12$ ,  $p > 0.05$ ), while the correlation between HGB and Trichostrongylidae was negative and highly significant, with medium magnitude ( $r=-0.16$ ).

Only in the resistant category was a negative correlation observed, with medium magnitude, between weight and WBC ( $r=-0.34$ ,  $p < 0.01$ ).



**Figure 2.** Mean values of the fecal egg count (FEC) (a) and packed cell volume (PCV) (b) of the 60 Morada Nova ewes, throughout the study (1 - 10 data collections over 6 months).

For WBC and Lymph, a negative correlation with medium magnitude was observed ( $r=-0.34$ ,  $p < 0.01$ ). The correlations of blood characteristics showed a negative correlation with high magnitude and high significance between Seg. Neut and Lymph ( $-0.74$ ,  $p < 0.01$ ), while Eosin and Lymph were negatively correlated with medium magnitude and high significance ( $r=-0.30$ ,  $p < 0.01$ ) (Figure 3). Finally, the correlation of Eosin count was negative with Trichostrongylidae ( $r=-0.11$ ,  $p > 0.05$ ).

### Resilient

For the resilient category, the correlation between weight and BCS was positive with high magnitude ( $r=0.68$ ,  $p < 0.01$ ), as were the correlations between BCS and PCV, and BCS and HGB ( $r=0.42$ ,  $r=0.56$ ,  $p < 0.01$ ) (Figure 4). The correlation between weight and PCV was positive and had medium magnitude ( $r=0.35$ ,  $p < 0.01$ ) just for this category.

The correlation of PCV with Trichostrongylidae ( $r=-0.24$ ,  $p < 0.01$ ) was negative with medium magnitude and significant. The correlation between HGB and Trichostrongylidae was negative, highly significant and had medium magnitude ( $r=-0.21$ ,  $p < 0.01$ ).

Among blood variables, the Lymph characteristic was negatively correlated with

Eosin, with medium magnitude and high significance ( $r=-0.22$ ,  $p < 0.01$ ), while between Seg. Neut and Lymph the correlation was negative with high magnitude and high significance ( $r=-0.83$ ,  $p < 0.01$ ).

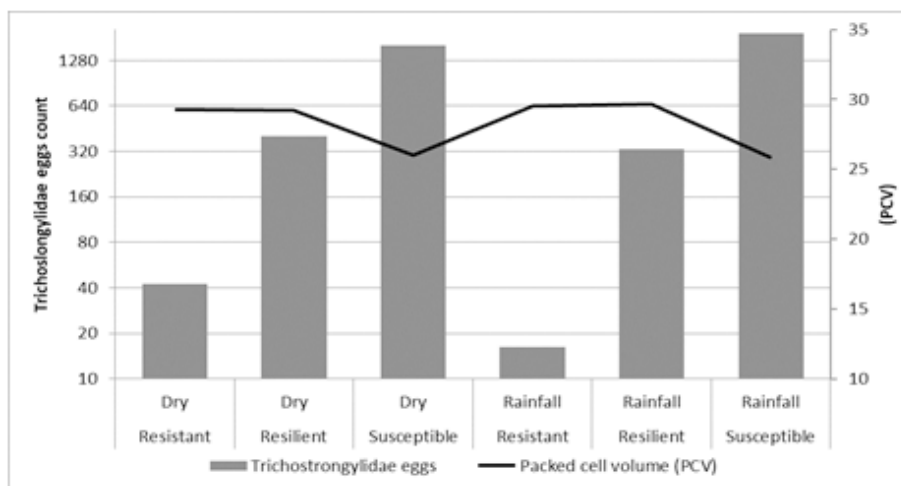
The correlation of Seg. Neut and Trichostrongylidae was positive ( $r=0.14$ ,  $p < 0.05$ ) and was negative between Eosin counts and Trichostrongylidae ( $r=-0.17$ ,  $p < 0.01$ ).

### Susceptible

The susceptible category showed the most differences in the correlation network. The weight and BCS correlations were positive and had medium magnitude ( $r=0.47$ ,  $p < 0.01$ ). The correlations were positive between BCS and PCV ( $r=0.45$ ) and BCS and HGB ( $r=0.35$ ), in both cases with high magnitude and high significance. The correlations of PCV with Trichostrongylidae and HGB with Trichostrongylidae were negative, with high magnitude and high significance ( $r=-0.56$ ,  $r=-0.51$ ,  $p < 0.01$ ).

The correlation between Lymph and Eosin was negative and had medium magnitude, but was not significant, and the correlation of Eosin count with Trichostrongylidae was negative but was close to zero and not significant ( $p > 0.05$ ).





**Figure 3.** Trichostrongylidae egg counts and packed cell volume of Morada Nova ewes resistant, resilient and susceptible during the dry and rainy seasons.

**Table I.** Means and standard deviations of weight, BCS and fecal egg count (FEC) of Morada Nova sheep according to category.

FEC						
Category	Weight	BCS	Trichostrongylidae	Strongyloides spp.	Moniezia spp.	Eimeria spp.
Resistant	31.5±3.6 <sup>a</sup>	2.19±0.6 <sup>a</sup>	29.9±83.7 <sup>a</sup>	0.39±6.2 <sup>a</sup>	0.77±12.5 <sup>a</sup>	60.5±216.9 <sup>a</sup>
Resilient	31.5±5.5 <sup>a</sup>	1.9±0.7 <sup>b</sup>	369.1±561.3 <sup>b</sup>	5.74±50.2 <sup>a</sup>	0±0.0 <sup>a</sup>	171.3±956.7 <sup>a</sup>
Susceptible	26.5±2.7 <sup>b</sup>	1.3±0.5 <sup>c</sup>	1756.9±1954.6 <sup>c</sup>	56.9±336.3 <sup>b</sup>	3.1±14.6 <sup>a</sup>	121.5±24.8 <sup>a</sup>

Means followed by different superscript letters in the same column differ by the Tukey test ( $p < 0.05$ ). Body condition score = BCS.

**Table II.** Means and standard deviations of packed cell volume (PCV), hemoglobin (HGB), red blood cells (RBC), white blood cells (WBC), segmented neutrophils (Seg Neut), lymphocytes (Lymph), eosinophils (Eosin) and monocytes (Mono) of Morada Nova ewes in São Paulo state, Brazil, according to the category.

Category	PCV (%)	HGB (g/dl)	RBC ( $\times 10^6/\mu\text{l}$ )	WBC ( $\times 10^3$ )	Seg. Neut (%)	Lymph (%)	Eosin (%)	Mono (%)
Resistant	29.4±3.5 <sup>a</sup>	7.76±0.7 <sup>a</sup>	9.4±1.0 <sup>a</sup>	6.03±1.6 <sup>a</sup>	42.8±13.0 <sup>a</sup>	39.3±13.1 <sup>a</sup>	16.6±8.5 <sup>a</sup>	0.9±1.2 <sup>a</sup>
Resilient	29.3±2.9 <sup>a</sup>	7.65±0.8 <sup>a</sup>	9.4±0.9 <sup>a</sup>	5.71±1.7 <sup>a</sup>	47.4±12.4 <sup>b</sup>	37.9±11.9 <sup>a</sup>	13.7±7.2 <sup>b</sup>	0.9±1.2 <sup>a</sup>
Susceptible	25.1±3.6 <sup>b</sup>	6.81±0.8 <sup>b</sup>	8.4±1.1 <sup>b</sup>	4.68±1.0 <sup>b</sup>	51.8±13.0 <sup>b</sup>	38.2±13.7 <sup>a</sup>	9.0±6.4 <sup>c</sup>	1.0±1.1 <sup>a</sup>

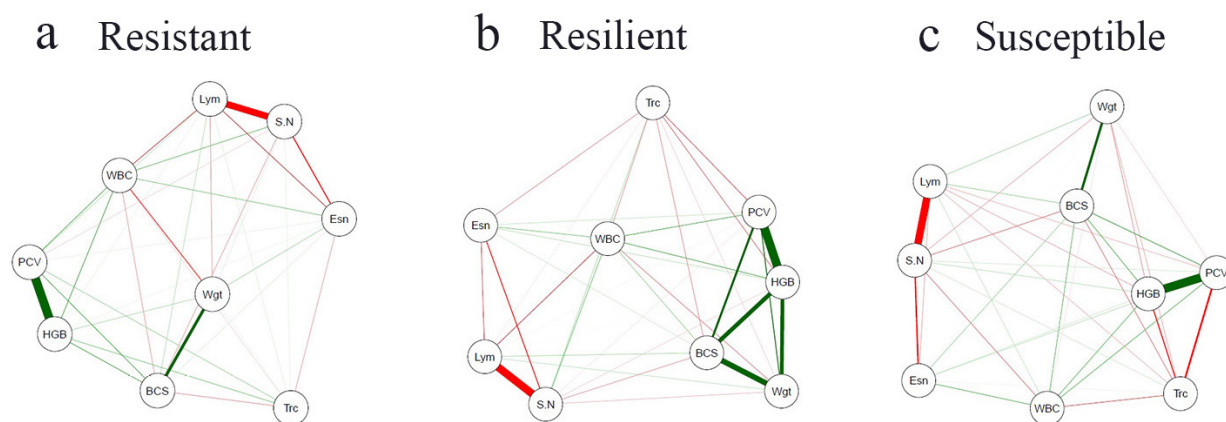
Means followed by different superscript letters in the same column differ by the Tukey test ( $p < 0.05$ ).

## DISCUSSION

The coproculture results showed no difference between the categories, with the predominance of *Haemonchus* spp. Many studies have shown that more than 80% of the parasite load of small ruminants is composed of *H. contortus*. (David et al. 2015, Bassetto & Amarante 2015, Nordi et

al. 2014), the main species that parasitizes sheep in regions with tropical and subtropical climate.

The environmental factor has a strong influence on the degree of infection, with rainy periods considered the most critical. In this period, the moist soil and rains favor the maintenance of fecal moisture, and consequently the development of larvae (Amarante et al.



**Figure 4.** Correlation network of resistant (a), resilient (b) and susceptible (c) categories: weight (Wgt), body condition score (BCS), packed cell volume (PCV), hemoglobin (HGB), white blood cells (WBC), segmented neutrophils (SN), lymphocytes (Lym), eosinophils (Esn) and Trichostrongylidae eggs (Trc) of Morada Nova ewes in São Paulo state, Brazil.

2014). However, even in the absence of rain, some larvae can move to plants. In this case, the moisture from dew may be sufficient to allow this migration (Santos et al. 2012), and in the dry season, the low supply of forage may affect the immunological response against parasites, a factor that can influence infection. However, in this study, no statistical differences were observed in the degree of infection between the seasons.

The maintenance of the parasitic infection and the percentage of PCV during the study are depicted in Figure 2, where the categories showed little variation in parasitic infection and phenotypic response that could be observed through blood parameters.

This can be explained by the age of the sheep and the exposure to natural infection for a long time, or due to their adaptability. These factors may have influenced the averages, causing the absence of statistical differences between the seasons. The susceptible category showed high infection in both seasons.

Throughout the evaluations, it was possible to observe that a small number of animals were highly infected, while most of the animals

maintained very low FEC or had no infection (Figure 1). The same behavior was observed under artificial infection in a study conducted with the same breed (Toscano et al. 2019). According to Sotomaior et al. (2007), this is due to the large individual difference in the ability to withstand the parasitic challenge.

The performance of the animals varied according to the categories, as did the parasitic loads. Usually the performance of animals that are facing challenge by pathogens is inferior to that of animals free from infection (Mavrot et al. 2015, Bishop & Stear 2003). The weight was statistically similar between the resistant and resilient categories, and both were different than the susceptible category. However, in this study weight was not sufficiently representative to be adopted alone as a characteristic for selection of parasitic resistance (Table I). The correlation coefficient between weight and Trichostrongylidae was low and not significant.

On the other hand, among the characteristics of the animals that can be analyzed only in the field, BCS better represented differences in animal performance than weight and was independent of the physiological condition,



since the averages among the categories were statistically different (Table I). Although the correlation coefficient between BCS and Trichostrongylidae was low, BCS obtained medium and high correlations with PCV and HGB for all categories. These blood parameters are considered clinical indicators of parasitic infection. Oliveira (2014), in a parasitological study, emphasized the importance of using the BCS parameter in the characterization of resistant and susceptible animals, reporting correlations between BCS and FEC and between hematological variables (PCV and HGB). Our results corroborate those findings.

There were discrepant differences in the degrees of infection of each category (Figure 1). The correlations between Trichostrongylidae and PCV and between Trichostrongylidae and HGB, in all categories, showed that as Trichostrongylidae increased, so did the degree of clinical anemia, caused mainly by the high prevalence of *H. contortus* (92%). The highest correlation coefficients were observed for the susceptible category ( $r=-0.56$  for PCV and  $r=-0.51$  for HGB). The low and non-significant correlations between Trichostrongylidae and PCV and between Trichostrongylidae and HGB in the resistant and resilient categories may have occurred due to their physiological responses to parasite infection. According to Costa et al. (2011), resistant animals have the capacity to prevent infection by parasites and even to eliminate them after ingestion, while resilient animals are capable of suffering no or low productive losses, as can be identified by hematological exams.

Afonso et al. (2010) reported low correlation between PCV and FEC and considered the possibility of negative correlations being attributed to sensitive animals, in line with our results.

The RBC values followed the PCV and HGB variation among the categories, with similar

values for resistant and resilient ( $9.4\pm 1.0\times 10^6/\mu\text{l}$  and  $9.4\pm 0.9\times 10^6/\mu\text{l}$ ) animals, respectively, and lower for susceptible ones ( $8.4\pm 1.2\times 10^6/\mu\text{l}$ ), with values slightly below the norm for adult sheep ( $9-15\times 10^6/\mu\text{l}$ ) (Carlos 2010). The lower values observed for susceptible animals attributed to pathogenicity caused by *H. contortus* is due to this parasite's feeding habit (hematophagous), causing significant reductions in hematological values due to the pathophysiology of the disease.

Correlation tests with RBC were not performed because it is a blood component directly associated with PCV and HGB, and these, in turn, better describe the clinical signs caused by infection by *H. contortus* (Issakowicz et al. 2016), and also because they are subject to greater variations due to physiological factors, such as hormonal actions (David et al. 2015).

The WBC averages were similar between resistant and resilient, and different from susceptible, with a lower average. The correlation between WBC and Trichostrongylidae was not significant in all categories, but for the resistant and resilient categories, the magnitudes were close to zero ( $r=-0.04$  resistant and  $r=0.03$  resilient), while for the susceptible group, the magnitude was  $r=-0.18$ .

The production of white blood cells is the first defense mechanism of the animal against infective agents. The differentiation of WBC is linked to the type of the host response to the etiologic agent. The correlation between WBC and Seg. Neut, significant only in the resistant category, can possibly be attributed to the immune response of the host when exposed to parasitic infection, whereas resilient and susceptible animals are constantly infected.

In ruminants, lymph cells are the most common cells. Studies have described the importance of lymphocyte response, differentiation of B lymphocytes into plasma

cells and the production of some isotypes and classes of antibodies, such as IgG1 and IgE (McRae et al. 2014), in the action against parasitic infections, as well as the action of eosinophils at the site of infection (Amarante et al. 2005). Currently, lymphocytes are the target of further studies by our group, to shed light on the immune system mechanism against parasitic infections.

The negative correlation with low and highly significant coefficients between Lymph and Eosin in the resistant and resilient categories suggests there is an immunological response with the possible participation of these two defense cells. In the susceptible category, there was low and negative correlation, but not significant, for these characteristics. It is not possible to affirm the action of these cells in the immunological response of each category, since the number of cells does not necessarily indicate functional activity; it only indicates phenotypic differences between the categories.

There was statistical difference in the means of Eosin between the categories. The ewes of the resistant group presented the highest mean of eosinophils, followed by the resilient and susceptible categories. This indicates the participation of these cells in the animals' defense against the parasites. During parasitic infection, defense cells migrate to the site of infection. Shakya et al. (2011) reported higher numbers of abomasal mucosal eosinophils, mast cells and neutrophils in infected compared to uninfected animals.

These findings agree with the observations of Amarante et al. (2007), who reported an inverse relationship between the number of inflammatory cells in the small intestine (mast cells, eosinophils, and globular leukocytes) and FEC values. Similarly, Stear et al. (2002) reported that the number of blood eosinophils circulating was higher in lambs with lower FEC.

The correlation between Eosin and Trichostrongylidae was significant only in the resilient category ( $r=-17$ ,  $p < 0.01$ ). The resistant category presented negative correlation with low magnitude and not significant ( $r = -11$ ), while the susceptible category presented a positive correlation close to zero and not significant. This fact may be associated with the migration of eosin to the infected area, thereby decreasing the amount of circulating eosin.

Buddle et al. (1992) observed a significant negative correlation between blood eosinophilia and FEC, which was related to the status of ovine resistance to parasites. According to those authors, eosinophilia is more associated with the expression of resistance against nematodes than as an indicator of infection, serving to evaluate the immune response. Therefore, eosinophil concentrations may be a useful indicator of resistance to parasite infection in animals that have been continually exposed to infection.

Using characteristics associated with parasitic infection, it was possible to identify resistant, resilient and susceptible sheep to *Haemonchus contortus*. The high percentage of resistant and resilient animals showed the low number of animals needing anthelmintic treatment, since the lowest percentage of animals were susceptible.

Trichostrongylidae eggs, BCS, PCV and eosinophil counts were found to be good indicators of naturally infected ewes and can be applied in procedures for *Haemonchus contortus* control.

The experimental evidence of this study showed that the phenotypic traits reported can be used to assess the resistance, resilience and susceptibility status of naturally infected Brazilian indigenous sheep.

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### **Author contributions**

David C.G.D - collected the data, developed the original hypotheses, designed the experiments and wrote the manuscript; Costa R.L.D. - collaborated in developing the original hypotheses, designing the experiments, interpreting the results and writing of the manuscript; Bartholazzi Junior A. - collaborated in interpreting of the results and producing the figures; Beltrame, R.T. and González A.R.M. - reviewed the manuscript; Madella-Oliveira A.F. - collaborated in interpreting the results; Quirino C.R. - conducted the statistical analyses and collaborated in interpreting the results.

