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Research article

EPIGENETIC INVESTIGATION RELATED TO GASTROINTESTINAL HELMINTH RESISTANCE AND PERFORMANCE IN CATTLE

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The objective was to characterize a herd of 73 Nellore heifers, identifying resistant, resilient, and susceptible animals to gastrointestinal helminths, relating the global methylation of the DNA of these animals with the degree of helminthiasis and factors that interfere with performance. Individual count of eggs per gram of feces (EPG), fecal culture for gender identification, weighing and blood sampling were carried out to determine PCV, STP, EOS, IgG, followed by DNA extraction and methylation analysis. The results were: 47% resistant animals, 34% resilient, and 19% susceptible to gastrointestinal helminth infections, with EPG counts of 53, 216, and 841, respectively, showing a statistical difference between all groups. The quantification of DNA methylation was 0.311, 0.245 and 0.178, respectively, for resistant, resilient, and susceptible animals, with a correlation being found between resistance to gastrointestinal helminths and overall DNA methylation. For weight gain, resistant and resilient animals showed higher values than susceptible ones, with a correlation between weight gain and EPG. The same was observed for VG; however, there was no statistical difference to the EOS, PPT, and IgG values. A significant correlation was found between PCV and EPG; quantifications of PCV and methylated DNA, STP and EPG; VG and STP. Therefore, the methodologies used made it possible to identify the animals regarding the degree of infection by gastrointestinal nematodes, making it possible to correlate the resistance of cattle to helminths with the amount of global DNA methylation and its performance.

Keywords: bovine, DNA methylation, parasitism, weight gain

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INTRODUCTION

The cattle herd in Brazil is of approximately 260,375,000 heads, which allows the country to occupy the 2nd position in the ranking of countries with the largest herds, being the largest commercial herd in the world [1].

Helminths are one of the main causes of poor production and growth, due to the reduction in productivity [2] as they present a negative impact on the development of these animals [3].

The use of anthelmintic drugs is the most used method to control helminth infections [4]. However, the indiscriminate use of these drugs can lead to the presence of anthelmintic resistance [5]. Thus, the search for alternatives to control these parasites is fundamental, such as the use of resistant animals. That is, animals that manage to suppress the establishment and/or development of these parasites [6-8]. Therefore, the selection of resistant animals can be highly efficient [9], as resistance is heritable [10].

Currently, there is research in the epigenetics field where the mechanisms involved in gene expression are studied [11], with these changes being heritable and reversible [12]. Among the most studied epigenetic mechanisms is DNA methylation [13], which often occurs in CpG islands [14] commonly present in the promoting gene regions, which when methylated and according to their location in the gene, influence the control of its expression and can be silenced, activated, or overexpressed [11,15,16]. Therefore, there is a change in the DNA methylation pattern, leading to the creation of a new profile, which has considerable effects on the animal's life and health [15].

Thus, the present study aims to characterize a herd of 73 Nelore heifers, identifying resistant, resilient, and susceptible animals to gastrointestinal helminths, relating the global DNA methylation of these animals to the degree of helminthosis and factors that interfere with performance.

MATERIALS AND METHODS

Ethical animal research

The study was approved by the Ethics Committee on Animal Use (CEUA) of Universidade Estadual Paulista (Unesp) (n. 09/2011.SP2).

Experimental design, area of study and animals

The study was carried out on a property located in the western region of the State of São Paulo - Brazil (20º51'27.8" S; 51º29'32.1" W), where 73 weaned contemporary Nellore cows, aging around eight months, from the same breeder lot, with an average initial weight of 173 kg. The animals received antiparasitic treatment

one month before the beginning of the evaluations, treated with 18,8% Levamisole Phosphate, following the manufacturer's recommended instructions for the control of gastrointestinal helminths and then remained with no antiparasitic treatment until the end of the experiment period.

Feces collection averages rated the degree of parasitism of the animals were performed every 28 days for 12 months, to later identify resistant, resilient, and susceptible animals according to the results obtained by counting eggs per gram of feces (EPG). Stool samples were collected directly from the rectal ampulla to perform EPG analysis using a McMaster according to the technique described [17], the feces were cultivated, and the larvae were extracted using the recommended technique [18] method for subsequent identification of the genera present [19]. Concomitantly with feces collection, every 28 days, all animals were weighed on a digital electronic scale to assess weight gain.

Every 84 days (one collection per season), 5 ml of blood were collected by phleboncentesis directly from the jugular vein for the determination of packed cell volume (PCV), serum total protein (STP), eosinophil (EOS) and immunoglobulins G (IgG). Another blood collection was also performed at the end of the study for later DNA extraction and methylation analysis. After collection, the material was placed in a transport refrigerator at 4°C until reaching the Laboratory of Parasitology and Animal Health (FCAT), being stored in a freezer (-20°C).

Extraction and Global DNA Methylation

The extraction of total genomic DNA was performed using the EasyPureBlood® Genomic DNA Kit (Transgen) and the quantification and quality of the samples were performed with the spectrophotometer (NanoDrop2000 – ThermoScientific), and the absorbance of each sample was measured in contrast to a buffer sample, at 260 and 280nm wavelengths [20]. The ratio between 1.8 and 2.0 is considered ideal, with values below 1.8 indicating protein excess and values above 2.0 indicating excess of organic solvents [21].

Methylation analysis was performed using the Imprint DNA Methylation Quantification kit (Sigma), using pools of pre-treated strips with methylated DNA binding reagent, and using a DNA methylation-sensitive capture antibody and a detection antibody, allowing the colorimetric detection of the relative amount of DNA methylation in relation to the positive control, which has 100% methylated DNA.

The absorbance of the solution contained in the wells, that is, the reading of the values, was performed at a wavelength of 450 nm in a DR-200BS-NM-BI microplate reader (MedLAb), and the formula used to obtain the absolute values (A450 Sample - A450 White).

Hematology and Serology

The determination of serum total protein (STP) was performed using the Strumia method [22]. The packed cell volume (PCV) and eosinophil count (EOS) were determined according to recommended techniques according to the Strumia method [22-24], respectively.

To evaluate the possible presence of antibodies to the infection caused by helminths, the quantification of the levels of IgG antibodies against L3 of *Haemonchus placei*, serum samples were used for the enzyme immunoassay that allows the detection of specific antibodies (ELISA), according to the described methodology by Cardoso et al. [25]. Standard *Haemonchus placei* positive serum samples were obtained from a previously infected Holstein calf [26]. These tests served as health indicators for the animals.

Statistical Analysis

Animal classification according to the degree of pasasitism

The distribution of EPG counts was verified by the Shapiro-Wilk test using the SISVAR 5.4 program [26] and the data transformed into $\sqrt[2]{n+0,5}$, where n is the value of the counts.

From the EPG counts in a completely randomized design, containing 73 treatments and 13 repetitions referring to each collection, the averages of each variable were submitted to analysis of variance (ANOVA) and subsequent statistical analysis of clustering by Tukey's test (5%), by the computer program SISVAR 5.4. Thus, the animals in the herd were, according to the degree of parasitism, grouped into three categories: resistant, resilient, and susceptible. After such classification, the mean EPG count of the animals in each group was also submitted to ANOVA, considering three treatments (classification according to resistance) and 73 repetitions (each animal being considered a repetition), and subsequent statistical analysis by the program computational SISVAR 5.4, by the Tukey test (5%) [26].

The statistical analysis of global DNA methylation, in a completely randomized design, used the absolute values of absorbance, being the averages of the methylation differences between the animals transformed into $\sqrt[2]{n+0,5}$ and submitted to ANOVA, by the computer program SISVAR 5.4, in order to verify the statistical significance between the different values of the resistant, resilient and susceptible groups to gastrointestinal helminths by the Tukey test (5%).

Other Statistical Analysis

The averages obtained from weight gain, PCV, EOS, STP and IgG were submitted to the variance test (ANOVA) and statistical analysis by the software SISVAR 5.4, and by Tukey test (5%).

To assess the relationship between the degree of helminthiasis and global DNA methylation, linear correlation analysis was performed between EPG and the global DNA methylation content using the Selegen-REML/BLUP Software for statistical analysis [27]. The same was also used to evaluate possible correlations between the other variables studied.

RESULTS

The degree of helminth infection dynamics, evaluated by the EPG count during the experimental period (Figure 1), indicates that all groups started the experiment with similar EPG averages, with an overall average of 13.69. However, throughout the study, susceptible animals showed a sharp and increasing increase in EPG (385 to 1100) in the sixth month, maintaining higher values when compared to resistant (73 to 38) and resilient (238 to 206) animals.

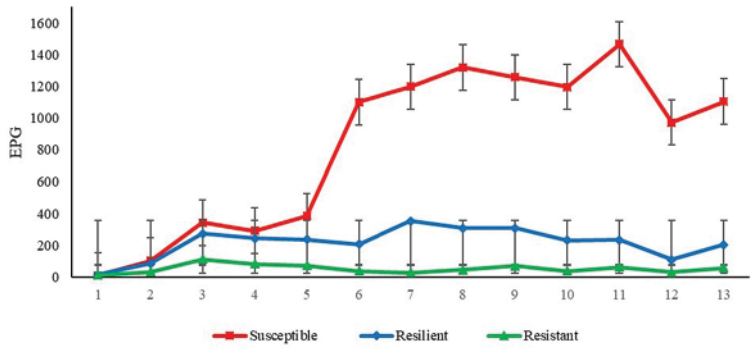


Figure 1. Overall average egg count per gram of feces (EPG) and ± standard error of the mean (SEM) of heifers throughout the experiment on heifers, distributed in resistant, resilient, and susceptible groups during the experimental period.

Table 1 shows the number of animals within each classification regarding the degree of infection by gastrointestinal nematodes, being: 34 resistant animals, 25 resilient animals and 14 susceptible animals, with EPG, which was respectively 53, 216 and 841, presenting an average overall of 260 EPG. For the degree of DNA methylation, resistant animals showed 0.311; resilient 0.245 and susceptible 0.178. Note that there was a significant difference between the global DNA methylation content of the three groups, also showing a significant negative correlation at 1% (-0.39) between the average EPG counts and the methylated DNA quantifications of the animals (Figure 3), with the resistant individuals presenting a greater amount of methylated DNA and the susceptible ones with a smaller amount. Resilient animals had an intermediate amount of methylated DNA.

Table 1. Summary of group sizes (N), average eggs per gram of feces (EPG), global DNA methylation content (measured as absolute absorbance values), initial EPG, initial and final weights, weight gain, and heifers categorized into resistant, resilient, and susceptible groups, coefficient of variation (CV) and p-value.

Classification	N	Average EPG	DNA Methylation	Initial EPG	Initial Weight	Final Weight	Weight Gain
Resistant	34	53 a	0,311 a	13,23	178,05	271,00 a	92,94 a
Resilient	25	216 b	0,245 ab	16,00	168,76	264,08 a	95,32 a
Susceptible	14	841 c	0,178 b	10,71	164,64	240,28 b	75,64 b
CV (%)		56,82	53,05	290,74	13,33	10,78	23,93
p-value		<0,001	<0,017	<0,001	<0,125	<0,004	<0,020

In Figure 2 it is possible to see that the animals of the three groups started the study with similar weights, presenting a general average of 172,3 kg, not differing statistically from each other. However, at the end of the experiment, when the final weight was recorded, the resistant and resilient animals presented higher weights in relation to the susceptible animals, these with weight gain of 17.3 kg and 19.7 kg less when compared to the resistant animals and resilient, respectively. Thus, regarding weight gain, there was no statistical difference between resistant and resilient, but both showed differences when compared to susceptible animals. When analyzing the relation between weight gain and EPG, a significant negative correlation was observed at 5% (-0.244) between the two variables (Figure 3).

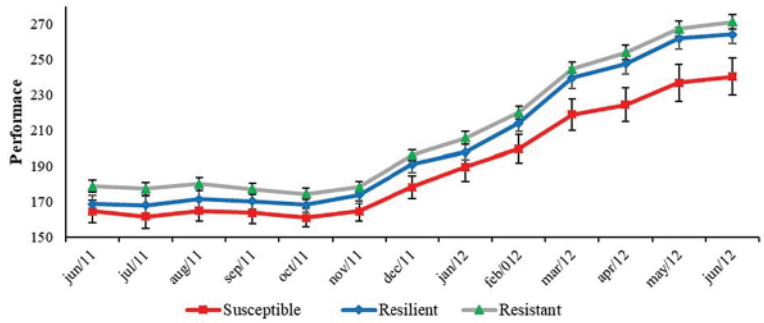


Figure 2. Average weight gain during and ± standard error of the mean (SEM) of heifers throughout the experiment on heifers, distributed in resistant, resilient, and susceptible groups during the experimental period.

The helminth genera found through fecal cultures showed a higher prevalence of *Cooperia* spp. (53.6%), followed by *Haemonchus* spp. (33.9%), *Oesophagostomum* spp. (12.3%) and *Trichostrongylus* spp. (0.2%).

For hematological analyses, it was observed that the PCV of resistant (37%) and resilient (36%) animals showed no significant difference, however, both differed from

susceptible animals (33%). In which animals showed significant negative correlations at 1% between PCV and EPG (-0.484). They also showed a significant correlation at 1% (0.324) between PCV and methylated DNA quantification of the animals. The mean values of EOS and STP in all groups showed no significant difference. However, there was a significant correlation at 5% between STP and EPG (0.232). A significant correlation at 1% (0.404) was also observed between PCV and STP (Figure 3).

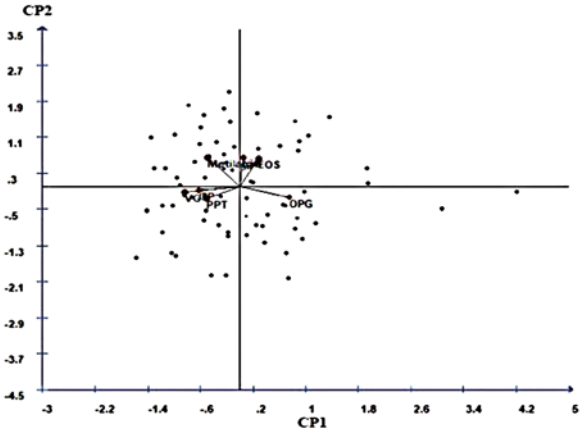


Figure 3. Scatterplot depicting the relationships among various variables, including global DNA methylation, eggs per gram (EPG), weight gain (WG), percent packed cell volume (PCV), eosinophils percentage (EOS), and serum total protein (STP).

Regarding the serological analysis evaluated through IgG data, it was noted that resistant (83.76%), resilient (70.58%) and susceptible (78.48%) animals did not differ statistically from each other. There was no correlation with any other variable studied (Table 2).

Table 2. Average values of percent packed cell volume (PCV), eosinophils percentage (EOS), serum total protein in grams per liter (STP), and immunoglobulin G percentage (IgG) in heifers categorized into resistant, resilient, and susceptible groups, coefficient of variation (CV), p-value and reference values.

Classification	N	PCV	EOS	STP	IgG
Resistant	34	37 a	13,56	70,3	83,76
Resilient	25	36 a	11,94	69,3	70,58
Susceptible	14	33 b	11	68,9	78,48
CV (%)		8	48,66	4,03	23,64
p-value		<0,001	<0,394	<0,430	<0,030
Reference Values		26 - 49	0 – 2.400	67,4 - 74,6	-

In Figure 3, it is possible to observe the results of the graphic dispersion of the variables studied, which demonstrates that the factor loadings of pressure in

the genotypes between global DNA methylation and EPG were different, as well as between WG and EPG, between EPG and PCV, between EPG, and STP. On the other hand, the factor loadings of pressure in the genotypes found between the variables PCV and STP, and between global DNA methylation and PCV, were equal.

DISCUSSION

The classification of heifers related to the degree of infection by gastrointestinal nematodes consents with Ueno and Gonçalves [28], in which they observed that average counts below 200 EPG represent a mild degree of infection, between 200 and 700 EPG represent a moderate degree and above 700 EPG represent a high degree of infection. In the present study, it can be observed that the mean EPG count of resistant animals was close to that found for Nellore animals, as reported by Bricarello et al. [29], who observed EPG counts below 50 for resistant animals.

For the overall average EPG count, it was observed that the values found in the present study were close to those found on steers (314 EPG) in the same region of the study [30] and above those reported for animals of the same genetic group [31]. This difference may be related to genetics [32,33] and to the challenge provided by the environment and climatic conditions of each region [31-34].

The distribution of the parasite load during the experimental period of resistant animals is lower, which indicates that the interaction between the parasite and the host's defense system can result in death and elimination of the worms [10]. As a consequence, there is an even more accentuated reduction in the contamination of pastures by infective larvae [35], which reduces the exposure of animals to the parasites and allows for an increase in productivity.

The negative correlation found between the variables weight gain and EPG, that is, the lower parasitic load of the animals provided for a better performance. As observed in studies with sheep, where the correlations found between EPG count and weight gain vary depending on the breed of animals and nematode species are generally negative [36-39].

It can also be verified that the category of susceptible heifers presented a high degree of infection, above 700 EPG [28]. This fact is explained because resistant animals have an immune response that limits the establishment of the parasite and in the case of resilience, animals are able to live with the parasites with minimal reduction in productivity [40], therefore, causing these groups of animals to gain weight more than susceptible animals.

Several studies since the 1980s indicate that a high parasite load promotes lower performance of animals kept on pasture, of approximately 30-70 kg less per year [41-43]. This corroborates the present study, where from the beginning in this research susceptible animals showed lower performance. These data are in agreement with the study by Neves [44], in which the parasitic action influenced the average

weight gain, reducing the performance of susceptible sheep compared to the resistant ones. As well as studies carried out by Bisset et al. [45], in which resistant Romney lambs were heavier than susceptible ones, and as Basseto et al. [46], in a study with Bergamácia ewes, in which the weight of resistant ewes was higher when compared to the weight of susceptible ewes.

Analyzing the fecal culture data, the most frequent species found in the Southeast and Midwest regions are *Cooperia punctata* and *Haemonchus placei* [46]. Soutello et al. [47] found that animals without anthelmintic treatment had a higher percentage of larvae of the genus *Haemonchus* spp., in relation to the genus *Cooperia* spp. This was not observed in any of the groups, as in the present study the cultures indicated a higher prevalence of larvae of the *Cooperia spp*., followed by *Haemonchus* spp., a result similar to the study carried out by Araujo and Lima [48] and Stromberg et al. [49], which in addition to the prevalence of the genus, the data obtained suggests that *C. punctata* has a deleterious effect both on appetite and on the absorption or utilization of nutrients, which compromises the weight gain of animals.

For the packed cell volume counts, it can be observed that the high parasite load in susceptible animals had a direct effect on the percentage of PCV, even though all groups were within normal values, which ranged from 26 to 49 [50]. Susceptible individuals had lower values for PCV when compared to the other groups. This can be explained by the fact that susceptible animals are the most affected in their parasitic load, in which some nematodes feed on blood and tissues [44], making this group the most affected. The negative correlation found between PCV and EPG demonstrates that animals with lower parasite load had higher PCV, as in studies by O'Kelly et al. [51] where low levels of infection by hematophagous parasites such as *Haemonchus* spp. verified in the animals of the present study, probably did not have a negative impact on PCV.

Concerning eosinophil counts, Neves [51] observed that sheep from resistant and susceptible groups did not differ significantly for average eosinophil counts, similar to what was found in the present study, where no significant difference was observed between groups. However, there are other studies where it is possible to notice that resistant animals have the highest eosinophil values, as for example, in a study carried out [49], in which the authors observed a significant difference in the number of eosinophils between a group of sheep considered resistant and a group considered susceptible, and the values were higher in resistant sheep.

All groups had serum total protein (STP) values within the normal range, which is of 67.4-74.6 g/L [52]. However, other authors report that values below what is considered normal are one of the consequences caused by haemonchosis, due to blood loss [53] caused by the spoliation produced by the parasites [29,53]. Also in the present study, a negative correlation was found between EPG and STP [54].

The fact that the animals presented IgG antigens in similar amounts in all groups against *Haemonchus placei* antigens corroborates studies carried out by Keus [55] where it was not possible to analyze the correlation between EPG values and serum IgG

levels, however, it is known that the humoral response is not the only factor involved in the immune response of ruminants against helminth infections. The presence of IgG antibodies in nematode infections is not valid for diagnosis, since cross-reaction between trichostrongylids is common, but it allows obtaining information on the dynamics of the immune response under experimental conditions [55].

There is only one report in the literature that relates bovine parasite resistance and global DNA methylation [47]. Few studies were found with production animals involving global methylation and other characteristics, such as: investigation of the occurrence of epigenetic events involved in anthelmintic resistance of *Haemonchus contortus* in sheep [57], blood DNA methylation relationship and performance of dairy cows [58] and analysis of the methylation profile of parasite resistance gene in horses [59].

However, in the present study, it was possible to correlate the quantification of methylated DNA and EPG, where animals with a higher degree of helminthiasis had a lower amount of methylated DNA, thus confirming the hypothesis studied. This corroborates the study carried out by Soutello et al. [47] with ½ Angus × ½ Nellore heifers, where a tendency for resistant animals to present a greater amount of methylated DNA was observed. A correlation was also observed between the quantification of methylated DNA and PCV, probably because the animals with higher parasite load and consequently higher EPG, presented lower values of PCV. Nematodes cause an increase in the degree of anemia in animals through the ingestion of blood and tissues from the gastrointestinal tract [60].

The results obtained in the study provide promising information for parasitology, due to the possibility that global methylation of blood DNA is linked to the expression of genes involved in parasite resistance, thus boosting studies on global methylation of bovine DNA and factors involved in the process of resistance to gastrointestinal helminths.

CONCLUSION

The methodologies used made it possible to identify the animals regarding the degree of infection by gastrointestinal helminths; resistance correlation was observed among cattle and helminths with the amount of global DNA methylation and its performance.

Data Availability Statement

The entire dataset supporting the results of this study was published in the article itself.

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Authors' contributions

IAC and MGFR carried out the molecular genetic studies, participated in the sequence alignment and drafted the manuscript. TMP carried out the immunoassays. GMF, TAC, MOM and FCP participated in the sequence alignment. BEP participated in the design of the study and performed the statistical analysis. DVFV and RVGS conceived of the study and participated in its design and coordination and helped to draft the manuscript. All authors read and approved the final manuscript.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

REFERENCES

1. Anualpec. Anuário da pecuária brasileira. São Paulo: IHS Markit - Agribusiness Intelligence; 2021, p. 35.
2. Oliveira PA, Ruas JL, Riet-Correa F, Coelho ACB, Santos BL, Marcolongo-Pereira C, Sallis ESV, Schild AL, Oliveira P, Ruas JL, RieT-Correa F, Coelho ACB, Santos BL, Marcolongo-Pereira C, Sallis ESV, Schild AL: Doenças parasitárias em bovinos e ovinos no sul do Brasil: frequência e estimativa de perdas econômicas. Pesq Vet Bras 2017, 37:797-801.
3. Stotzer ES, Lopes LB, Eckstein C, Moraes MCMM, Rodrigues DS, Bastianetto E: Impacto econômico das doenças parasitárias na pecuária. Revista Brasileira de Higiene e Sanidade Animal 2014, 8:198–221.
4. Brito LG, Barbieri FS, Rocha RB, Santos APL, Silva RR, Ribeiro ES, Guerrero F, Foil L, Oliveira MCS: Pyrethroid and organophosphate pesticide resistance in field populations of horn fly in Brazil. Med Vet Entomol 2019, 33:121–130.
5. Soutello RVG, Seno MCZ, Amarante AFT: Anthelmintic resistance in cattle nematodes in northwestern São Paulo State, Brazil. Vet Parasitol 2007, 148: 360–364.
6. Utech KBW, Wharton RH, Kerr JD: Resistance to *Boophilus microplus* (Canestrini) in different breeds of cattle. Aust J Agric Res 1978, 29:885–895.
7. Suarez VH, Busetti MR, Lorenzo RM: Comparative effects of nematode infection on Bos taurus and Bos indicus crossbred calves grazing on Argentina's Western Pampas. Vet Parasitol 1995, 58:263–271.
8. Gasbarre LC, Leighton EA, Sonstegard T: Role of the bovine immune system and genome in resistance to gastrointestinal nematodes. Vet Parasitol 2001, 98:51–64.
9. Biegelmeyer P, Nizoli LQ, Cardoso FF, Dionello, NJL: Aspectos da resistência de bovinos ao carrapato *Rhipicephalus* (Boophilus) *microplus*. Archivos de zootecnia 2012, 61:1–11.

10. Amarante AFT: Resistência genética a helmintos gastrintestinais. In: Simpósio da Sociedade Brasileira de Melhoramento Animal, 2004, Pirassununga–SP. Anais V Simpósio da Sociedade Brasileira de Melhoramento Animal, 2004, 1:10.
11. Rivas MP, Teixeira ACB, Krepischi ACV: Epigenética: conceito, mecanismos e impacto em doenças humanas. Genética na Escola 2019, 14:14–25.
12. Blomen VA, Boonstra J: Stable transmission of reversible modifications: maintenance of epigenetic information through the cell cycle. Cell and Mol Life Sci 2011, 68:27–44.
13. Niciura SCM, Moraes CV, Cruvinel GG, Gainza YA, Albuquerque ACA, Santana RCM, Tholon P, Tizioto PC, Chagas ACS, Esteves SN, Benavides MV, Amarante AFT: Análise genômica da resistência ao monepantel e investigação epigenética em *Haemonchus contortus*. São Carlos: Embrapa Pecuária Sudeste; 2018, 5-14.
14. Paiva JT, Resende MDV, Resende RT, Oliveira HR, Silva HT, Caetano GC, Lopes P S, Silva FF: Epigenética: mecanismos, herança e implicações no melhoramento animal. Archivos de zootecnia 2019, 68:304–311.
15. Fantappie M: Epigenética e memória celular. Revista Carbono 2013, 3:1–5.
16. Reis AP: Epigenética da asma: revisão. Arquivos de Asma, Alergia e Imunologia 2015, 3:13–18.
17. Rashid, MH, Stevenson MA, Waenga S, Mirams G, Campbell AJD, Vaughan JL, Jabbar A: Comparison of McMaster and FECPAKG2 methods for counting nematode eggs in the faeces of alpacas. Parasites Vectors 2018, 11:1–4.
18. Robert FHS, O'Sullivan PJ: Methods for eggs counts and larval cultures for Strongyles infecting the gastrointestinal tract of cattle. Aust J Agric Res 1950, 1:99–102.
19. Keith R K. The differentiation of the infective larvae of some common nematode parasites of cattle. Aust J Zoo 1953, 1: 223–235.
20. Ferreira ME, Grattapaglia D. Introdução ao uso de marcadores moleculares em análise genética. Brasília: Embrapa-Cenargen; 1998, 11-204.
21. Wolf AV, Fuller JB, Goldman EJ, Mahony TD: New refractometric methods for determination of total proteins in serum and in urine. Clinical Chemistry 1962, 8:158–165.
22. Strumia MM, Sample AB, Hart ED: An improved microhematocrit method. Am J Clin Pathol 1954, 24:1016–1024.
23. Schalm OW, Carroll EJ. Veterinary hematology. In: Philadelphia: Lea & Febiger;1986, 1277.
24. Cardoso CP, Silva BF, Trinca LA, Amarante AFT: Resistance against gastrointestinal nematodes in Crioulo Lageano and crossbred Angus cattle in southern Brazil. Vet Parasitol 2013, 192:183–191.
25. Bassetto CC, Silva BF, Newlands GFJ, Smith WD, Amarante AFT: Protection of calves against *Haemonchus placei* and *Haemonchus contortus* after immunization with gut membrane proteins from *H. contortus*. Parasite Immunol 2011, 33:377–381.
26. Ferreira DF. Sisvar: a Guide for its Bootstrap procedures in multiple comparisons. Ciênc Agrotec 2014, 38:109–112.
27. Resende MDV: Selegen–Reml/Blup Sistema estatístico e seleção genética computadorizada via modelos lineares mistos. Colombo: Embrapa Florestas; 2007, 22-351.
28. Ueno H, Gonçalves PC: Manual Para Diagnóstico das Helmintoses de Ruminantes. Japan International Cooperation Agency; 1998, 1-135.
29. Bricarello PA, Zaros LG, Coutinho LL, Rocha RA, Kooyman FNJ, De Vries E, Gonçalves JRS, Lima LG, Pires AV, Amarante, AFT: Field study on nematode resistance in Nelore-breed cattle. Vet Parasitol 2007, 148:272–278.

30. Soutello RVG, Condi GL, Paes F, Fonzar JF: Influência do Parasitismo e da Suplementação Proteica no Desenvolvimento Ponderal de Novilhos Mestiços Angus-Nelore e da Raça Guzerá. Ciências Agrárias e da Saúde 2002, 2:21–27.
31. Oliveira MCS, Alencar MM, Chagas ACS, Giglioti R, Oliveira HN: Gastrointestinal nematode infection in beef cattle of different genetic groups in Brazil. Vet Parasitol 2009, 166:249–254.
32. Leighton EA, Murrell KD, Gasbarre LC: Evidence for Genetic Control of nematode egg-shedding rates in calves. J Parasitol 1989, 75:498–504.
33. Passafaro TL, Carrera JPB, Santos LL, Raidan FSS, Santos DCC, Cardoso EP, Leite RC, Toral FLB: Genetic analysis of resistance to ticks, gastrointestinal nematodes and *Eimeria spp.* in Nellore cattle. Vet Parasitol 2015, 210:224–234.
34. Pimentel MN, Fonseca AH: Epidemiologia das helmintoses pulmonares e gastrintestinais de bezerros em região de baixada do Estado do Rio de Janeiro. Pesq Vet Bras 2002, 22:148–152.
35. Barger IA: Genetic resistance of hosts and its influence on epidemiology. Vet Parasitol 1989, 32:21–35.
36. Bishop SC, Bairden K, Mckellar QA, Park M, Stear MJ: Genetic parameters for faecal egg count following mixed, natural, predominantly Ostertagia circumcincta infection and relationships with live weight in young lambs. Anim Sci 1996, 63:423–428.
37. Bisset SA, Vlassof A, Morris CA, Southey BR, Baker RL, Parker AGH: Heritability of and genetic correlations among fecal egg counts and productivity traits in Romney sheep. New Zealand J Agric Res 1992, 35:51–58.
38. Eady SJ, Woolaston RR, Ponzoni RW, Lewer RP, Raadsma HW, Swan AA: Resistance to nematode parasites in Merino sheep: correlation with production traits. Aust J Agric Res 1998, 49:1201–1212.
39. Bouix J, Krupinski J, Rezepecki R, Nowosad B, Skrzyzala I, Roborzynski M, Fudalewicz-Niemczyk W, Skalska M, Malczewski A, Gruber L. Genetic resistance to gastrointestinal n ematode parasites in Polish long-wool sheep. Int Parasitol 1998, 28:1797–1804.
40. Albers GA, Gray GD, Piper LR. Barker JS, Le Jambre LF, Barger IA: The genetics of resistance and resilience to *Haemonchus contortus* in young merino sheep. Int J Parasitol 1987, 17:1355–1363.
41. Pinheiro AC, Alves-Branco FPJ, Sapper MFM: Impacto econômico das parasitoses nos países do Mercosul. In: Seminário Brasileiro de Parasitologia Veterinária, 11, Salvador. Anais. Salvador, Colégio Brasileiro de Parasitologia Veterinária 1999, 59–60.
42. Zocoller MC, Starke WA, Valério Filho WV: Ganho de peso em fêmeas da raça Guzerá tratadas com diferentes épocas de aplicação de anti-helmínticos. In: Seminário Brasileiro de Parasitologia Veterinária, 9., Campo Grande. Anais. Campo Grande: CBPV 1985, 124.
43. Bianchin I, Honer MR, Nunes SG, Nascimento MA, Curvo JB, Costa FP: Epidemiologia dos nematódeos gastrintestinais em bovinos de corte nos cerrados e o controle estratégico no Brasil. Campo Grande: Embrapa Gado de Corte; 1993, 113.
44. Neves MRM: Utilização de marcadores fenotípicos para caracterização de ovinos mestiços Santa Inês naturalmente infectados com nematóides gastrintestinais. Sobral: Embrapa Sudeste; 2010.
45. Bisset SA, Morris CA: Feasibility and implications of breeding sheep for resilience to nematode challenge. Int J Parasitol 1996, 26:857–868.

46. Basseto CC, Silva BF, Fernandes S, Amarante AFT: Contaminação da pastagem com larvas de nematoides gastrintestinais após o pastejo de ovelhas resistentes ou susceptíveis à verminose. Rev Bras Parasitol Vet 2009, 18:63–68.

47. Soutello RVG, Rodrigues MGF, Gonçalves JA, Bello HJS, Pavan BE, Ramos ES: Global genomic methylation related to the degree of parasitism in cattle. Sci Rep, 2022, 12:18135.

48. Araujo RN, Lima WS: Infecções helmínticas em um rebanho leiteiro na região Campo das Vertentes de Minas Gerais. Arq Bras Med Vet e Zootec 2005, 57: 186–196.

49. Stromberg BE, Gasbarre LC, Waite A, Bechtol DT, Brown MS, Robinson NA, Olson EJ, Newcomb H: Cooperia punctata: effect on cattle productivity. Vet Parasitol 2012, 183: 284–291.

50. Coles GC, Bauer C, Borgsteede FH, Geerts S, Klei TR, Taylor MA, Waller PJ: World association for the advancement of veterinary parasitology (WAAVP) methods for the detection of anthelmintic resistance in nematodes of veterinary importance. Vet Parasitol 1992, 44:35–44.

51. O'Kelly JC, Post TB, Bryan RP: The influence of parasitic infestations on metabolism, puberty and first mating performance of heifers grazing in a tropical area. Anim Reprod Sci 1988, 16:177–189.

52. Kaneko JJ, Harvey WJ, Brus LM: Bioquímica Clínica de Animais Domésticos. Academic Press, Cambridge, MA 1997.

53. Amarante AFT: Controle de verminose ovina. Revista CFMV 2005, 34:19–30.

54. Oliveira PA, Ruas JL, Riet-Correa F, Coelho ACB, Santos BL, Marcolongo-Pereira C, Sallis ESV, Schild AL, Oliveira PA, Ruas JL, RieT-Correa F, Coelho ACB, Santos BL, Marcolongo-Pereira C, Sallis ESV, Schild AL: Doenças parasitárias em bovinos e ovinos no sul do Brasil: frequência e estimativa de perdas econômicas. Pesq Vet Bras 2017, 37:797–801.

55. Kanobana K, Ploeger HW, Vervelde L: Immune expulsion of the trichostrongylid *Cooperia oncophora* is associated with increased eosinophilia and mucosal IgA. Int J Parasitol 2002, 32:1389–1398.

56. Cuquerella M, Gómez-Muñhoz MT, Carrera L, Fuente C, Alunda JM: Cross antigenicity among ovine trichotrongyloidea. Preliminary report. Vet Parasitology 1994, 53:243–251.

57. Niciura SCM, Veríssimo CJ, Gromboni JGG, Rocha MIP, Mello SS, BarbosaCMP, Chiebao DP, Cardoso D, Silva GS, Otsuk IP, Pereira JR, Ambrosio LA, Nardon RF, Ueno TEH, Molento MB: F200Y polymorphism in the β-tubulin gene in field isolates of Haemonchus contortus and risk factors of sheep flock management practices related to anthelmintic resistance. Vet Parasitol 2012, 190:608–612.

58. Wang L, Su, HZ, Guan LL, Liu JX: Short communication: Relationship of blood DNA methylation rate and milk performance in dairy cows. J Dairy Sci 2019, 102:5208–5211.

59. Pires VS, Ganzella FAO, Minozzo, Castro LLD, Moncada ADB, Klassen G, Ramos EAS, Molento MB: Epigenetic regulation of SLC11a1 gene in horses infected with cyathostomins. Gene reports 2021, 25:101410.

60. Jimenez-Sanz A, Quirino CR, Pacheco A, Costa RLD, Costa RLD, Beltrame RT, Rua MAS, Silva RMC, Madella-Oliveira AFM: Relação entre fatores associados às parasitoses gastrointestinais, desempenho e estado fisiológico de ovelhas Santa Inês. Agrotec 2016, 37:88–95.

EPIGENETIČKA ISTRAŽIVANJA U VEZI SA RESISTENCIJOM GASTROINTESTINALNIH HELMINTA I PERFORMANSAMA KOD GOVEDA

Isabela de Almeida CIPRIANO, Giordani Mascoli de FAVARE, Tábata Alves do CARMO, Mateus Oliveira MENA, Daniel Vitor Ferreira VICARI, Fernanda Calazans PAGNOZZI, Tiago Marolato PACHECO, Maria Gabriela Fontanetti RODRIGUES, Bruno Ettore PAVAN, Ricardo Velludo Gomes de SOUTELLO

Cilj je bio da se okarakteriše stado od 73 Nellore junica, identifikujući rezistentne, otporne i osetljive životinje na gastrointestinalne helminte, povezujući globalnu metilaciju DNK ovih životinja sa stepenom helmintijaze i faktorima koji ometaju performanse. Individualno prebrojavanje jaja po gramu fecesa (EPG), fekalna kultura za identifikaciju pola, merenje i uzimanje uzoraka krvi su obavljeni u cilju određivanja PCV, STP, EOS iIgG. Nakon navedenog usledila je ekstrakcija DNK i analiza metilacije. Rezultati su bili: 47% rezistentnih životinja, 34% otpornih i 19% podložnih infekcijama gastrointestinalnim helmintima, sa odgovarajućim EPG brojem od 53, 216 i 841, što pokazuje statističku razliku između svih grupa.

Kvantifikacija metilacije DNK bila je 0,311, 0,245 i 0,178, za rezistentne, otporne i osetljive životinje, pri čemu je pronađena korelacija između rezistencije na gastrointestinalne helminte i ukupne DNK metilacije. Za povećanje telesne težine, rezistentne i otporne životinje su pokazale veće vrednosti od osetljivih, uz korelaciju između povećanja telesne težine i EPG. Isto je primećeno i za VG; međutim, nije bilo statističke razlike u vrednostima EOS, PPT i IgG. Utvrđena je značajna korelacija između PCV i EPG; kvantifikaciju PCV i metilovane DNK, STP i EPG; VG i STP. Stoga su korišćene metodologije omogućile identifikaciju životinja u pogledu stepena zaraze gastrointestinalnim nematodama, što je omogućilo korelaciju otpornosti goveda na helminte sa količinom globalne metilacije DNK i njenim performansama.