Unraveling the genetic variability of host resilience to endo and ectoparasites under natural infestation, in Nellore cattle

Gabriela Canabrava Gouveia¹, Virgínia Mara Pereira Ribeiro¹, Marina Rufino Salinas Fortes²-³, Fernanda Santos Silva Raidan⁴, Antonio Reverter-Gomez⁵, Mariana Mamedes de Moraes¹, Andresa Eva Melo de Araújo¹, Daniel Resende Gonçalves⁶, Marcos Vinicius Gualberto Barbosa da Silva⁷, Fabio Luiz Buranelo Toral¹*

¹Departamento de Zootecnia, Escola de Veterinária, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil.
²School of Chemistry and Molecular Bioscience, The University of Queensland, Brisbane, Queensland, Australia.
³Queensland Alliance for Agriculture and Food Innovation, The University of Queensland, Brisbane, Queensland, Australia.
⁴Agriculture and Food, Commonwealth Scientific and Industrial Research Organization (CSIRO) – Hobart, Tasmania, Australia.
⁵Agriculture and Food, Commonwealth Scientific and Industrial Research Organization (CSIRO) – Brisbane, Queensland, Australia.
⁶Mundo Novo farm, Uberaba, Brazil.
⁷Embrapa Gado de Leite, Empresa Brasileira de Pesquisa Agropecuária, Juiz de Fora, Brazil.

*Correspondence:
Gabriela Canabrava Gouveia

Email addresses: gabrielacgouveia@hotmail.com, virginiamara16@gmail.com, m.fortes@uq.edu.br, Fernanda.Raidan@csiro.au, Toni.Reverter-Gomez@csiro.au, mamedesm@hotmail.com, andresamelomg@hotmail.com, drgoncalves@fazendamunandonovo.com, marcos.vb.silva@embrapa.br, flbtoral@ufmg.br.
Abstract

Background:
Host resilience (HR) to parasites can affect growth in pastured raised cattle. This study is a detailed investigation of the genetic mechanisms of HR to ticks (TICK), gastrointestinal nematodes (GIN), and Eimeria spp. (EIM) under natural infestation. HR was defined as the slope coefficient of random regression models of body weight (BW) when TICK, GIN, and EIM burdens were used as environmental gradients. The BW was evaluated in five measurement events (ME): when animals were 331, 385, 443, 498, and 555 days old on average. 7307 BW records were available from 1712 animals weighted at least in one ME. Out of those, 1075 animals had valid genotypic information after quality control analysis that were used in genome-wide association studies (GWAS) and GWAS meta-analyses to identify genomic regions associated with HR.

Results:
Both the genetic correlations between intercept and HR to each parasite, and the genetic correlations between BW measured in animals submitted to different parasite burden indicated that there was genotype x parasite burden interaction for BW, and selection for BW under environment with controlled parasite burden might be an efficient strategy to improve both, BW and HR. Furthermore, there was no impact of age of measurement on genetic variance estimates for HR to different parasites. However, genetic correlation between HR to the same parasite measured in different ages ranged from low to moderate in magnitude, with a posteriori means (high posterior density interval with 90% of samples) varying from 0.13 (-0.05; 0.35) to 0.40 (0.15; 0.63) for TICK, from 0.11 (-0.06; 0.29) to 0.52 (0.37; 0.67) for GIN and from 0.25 (0.07; 0.43) to 0.56 (0.34; 0.77) for EIM. These results indicate the importance of age of measurement in studies on HR.

Conclusions:
HR to GIN and EIM can be used as a complementary tool to parasitic control management, and a multiple trait selection method that combine BW and HR to parasites should be used in parasitic endemic areas to avoid economic losses due parasitic diseases.

Keywords: ticks, gastrointestinal nematodes, genetic parameters, Eimeira spp., random regression models, response to disease, parasitic disease, GWAS
1 Background

Ecto and endoparasites such as ticks (TICK), gastrointestinal nematodes (GIN), and *Eimeria* spp. (EIM) are endemic in tropical countries and they are also responsible for several economic and productivity losses in cattle production systems [1]. Moreover, parasitic loads represent an important challenge to the sustainability of cattle production, especially in tropical countries, such as Brazil.

The negative impact of ticks on cattle production is due to the direct effects of feeding, such as weight loss, anaemia, and damage of leather, and indirect effects, such as the transmission of tick-borne pathogens [2]. Gastrointestinal parasites like GIN and EIM also negatively affect the cattle performance due to both direct and indirect effects: competition for nutrients, physical tissues damage, and the host immune response to parasite invasion [3–5]. Reduced food intake, weight loss, diarrhea, and dehydration are the main symptoms of these intestinal parasitosis [4,6,7]. For EIM infections, anaemia is also an important symptom [3].

The sustainability of cattle production in endemic areas depend on the animal’s ability of respond to stressor factor as parasite loads. The terminology around the different mechanisms of host’s response to disease is still confuse. In general, animals can respond to disease using two complementary mechanisms: resistance and tolerance [8]. The host resistance can be characterized by the ability of a host to limit parasite burdens while host tolerance can be defined as the ability to limit the damage caused by a given parasite burden (Råberg et al., 2009). Therefore, the host resilience (HR) is the phenotype that captures these two mechanisms against pathogens [8,9], and can be defined as the animal capability of maintain a relatively undepressed production level when subjected to environmental parasite burdens [10,11].

The HR can be estimated as a continuous trait using reaction norm models of performance on environmental parasitic load [8]. In this case, the additive variance of dependent variable, is divided into two coefficients: the intercept that is the additive component of the variability in performance and the slope that is the HR [12]. Moreover, when linear regressions are used, the genetic correlation between the intercept and slope coefficients quantify the genetic association between host fitness and HR [13]. Significative correlations between these two parameters indicate, thus, the presence of genotype x environmental interaction [14].

Reaction norm models have been used to estimate HR in milk production and fertility traits to *Fasciola hepatica* in Irish cattle [15]. The authors used the prevalence of *Fasciola hepatica* in each herd and year to define the environmental gradient. In our study, we estimated HR in body weight to TICK, GIN, and EIM burdens to estimate genetic parameters of HR in beef cattle. The slope solutions were considered as the estimated breeding values for HR and used as a phenotype to perform genome-wide association studies (GWAS) for HR to each parasite. Therefore, the main objectives of the present study are to estimate genetic parameters for HR of cattle to different parasites, evaluate the trends of these traits with aging, and suggest possible mechanisms influencing HR to parasites in Nellore cattle.

In short, this study is an investigation of the genetic mechanisms of HR to endo and ectoparasites under natural infestation. The genetic parameters of HR and its association with BW uncovered here are evidence for discussing the inclusion of HR as selection criteria in cattle breeding programs.

2 Material and Methods

2.1 Data collection and edition
2.1.1 Data collection

The phenotype and genotype data from Nellore bulls, born between 2010 and 2016 and raised in a commercial farm named Mundo Novo, located in Uberaba, Minas Gerais state, Brazil (19° 24' 33" S and 48° 06' 34" W, altitude of 840 meters, Monsoon-influenced humid subtropical climate or Cwa weather according to Köppen scale) were used in this study. Only animals that were born and raised in the same farm were considered in the present study. The Ethics and Animal Experimentation Committee of the Universidade Federal de Minas Gerais approved the experiment and data collection (Protocol 255/2010).

The bulls were pasture raised, with pasture formed mainly (>80%) by grass of Uruchloa genus, with a stocking rate of approximately 0.98 animal unit per hectare (one animal unit is equivalent to 450-kg). Animals had free access to mineral supplementation and clean water throughout the year. After weaning (210 days old on average), the males were arranged into groups of around 45 animals with age range of 90 days in each group. These animals were evaluated in performance tests that lasted 294 days, being 70 days of adaptation and 224 days of evaluation (Figure 1). The adaptation period is required because animals are submitted to nutritional stress (changes in mineral supplementation), and social stress (they were weaned, and new groups were formed according to age).

The bulls were weighed at six measurement events (ME): at day 1 of the performance test, at the end of the adaptation period (day 70) and in intervals of 56 days until the end of the test. BW information registered at day 1 was not used in this study. The intervals of 56 days defined five ME. The animals were on average 331, 385, 443, 498, or 555 days old in each of the five ME.

The parasite counts used in the present study were obtained at each ME through counts of engorged female ticks (length size > 4.5 mm - TICK) on the right side of each animal. The length size of the engorged female ticks were defined according to the technique proposed by Wharton and Utech [16]. Furthermore, fecal samples were collected directly from the animals' rectum using properly identified and lubricated plastic bags to proceed to the count of gastrointestinal parasites. The fecal samples were cooled and transferred in chilled coolers to the Laboratory of Parasitic and Mycotic Diseases of the Escola de Veterinária - Universidade Federal de Minas Gerais (EV-UFMG). In the laboratory, the counting of eggs of gastrointestinal nematodes (GIN) and oocysts of Eimeria spp. (EIM) per gram of feces were processed, according to the modified McMaster technique [17]. To perform the counts, we diluted 2g of faeces with 28ml of water, aliquoted 2ml of this mixture and mixed with 2ml of saturated Sheater’s solution (500 g of sugar, 6.5 ml of phenol and 360 ml of water). Then, 0.15ml of the final solution was used to fill the McMaster chamber used to perform the counts for eggs and oocysts.

There is a practice of multiplying tick counts by two to estimate the tick burden in the entire animal [18]. For the modified McMaster technique, a multiplicative factor can also be used to infer on the animal’s parasite burden. This multiplicative factor depends on the dilution used to prepare the test. For the dilution we used, the adequate multiplicative factor is 50 [19,20]. Ticks, eggs, and oocysts counts used in the present study are the real counts observed on the right side of each animal or at the McMaster chamber, without multiplication by any constant. The bulls included in the present study were subjected to natural parasite infestation because they were raised in a herd with commercial purposes. Approximately 65% of the bulls were dewormed at the beginning of the performance tests (day 1 of adaptation period) with Ivermectin 4% (1ml of Ivermectin per 50Kg of live BW - Master LP, Ouro Fino Saúde Animal, Cravinhos, SP). The dewormed bulls were randomly chosen by contemporary group, in such a way that we had some entire groups dewormed or not. The contemporary groups were defined as the group of animals that were raised together in the same paddock.
Blood samples were collected with sterilized syringes into vacuum tubes of 3.5 ml containing 9NC Coagulation Sodium Citrate 3.2%, to conserve the host’s DNA. Blood samples were frozen and transferred in chilled coolers to the Laboratory of Genetics at EV-UFMG, where they were stored in freezers at -20°C. 1230 blood samples were selected for genotyping with a low-density DNA array: the Z-chip v2 (Neogen, Lincoln, Nebraska, EUA, which genotypes – 27533 SNPs). Most of the genotyped bulls were from the performance tests with more than 20 animals per group, as described above, and each animal had information regarding the three parasites, in at least four ME. Some genotyped animals did not have phenotypic measures, but they were representative sires of this herd (31 genotyped sires without phenotypic records with an average of 25.26 offspring in the relationship matrix).

2.1.2 Phenotypic data editing

The data set of phenotypes had information on BW, TICK, GIN, and EIM. For each ME we have only considered animals that had information regarding the four phenotypes. Cohorts were defined by the combination of contemporary group and ME. Bulls belonging to cohorts with less than five animals were not considered in the study. At the end of the data editing process, 1712 animals had information in at least one ME (Table S1). These animals were offspring of 130 sires (with 13.17 ± 12.46 offspring - mean ± standard deviation) and 1132 cows (1.51 ± 0.77 offspring). The relationship matrix was formed by 5933 animals. The summary statistics of the phenotypes used in the present study are presented in Table 1, phenotypes’ distributions are presented in Supplementary Figure 1.

2.1.3 Genotypic data editing

Blood samples of 1230 animals were collected according to the criteria described above. The quality control of DNA samples and markers was carried using the SNP & Variation Suite v8.8.3 software [21]. Alleles with a GenTrain Score < 0.6 were considered as missing calls in the panel. Only SNPs with call rate ≥ 0.95, minor allele frequency ≥ 0.05, and located at the autosome and X chromosomes, and samples with call rate > 0.90 were analyzed. After the quality control procedure, the SNP panel was formed by 21667 SNPs (78.7% of all tested SNPs) and 1075 samples (87.4% of genotyped samples).

2.2 Covariance components

2.2.1 Environmental parasite burden

The environmental pathogen load is a crucial component of resilience that must be considered [8]. Thus, we used the median counts of TICK, GIN, and EIM in each cohort as the environmental gradient, that is formed by the combination of animals raised in the same paddock (contemporary groups) on the same period of the year (ME). Each combination of parasite x ME was used to generate a different dataset, for which the genetic parameters were estimated. Cohorts with median parasite count equal to zero do not indicate they are parasite free cohorts because at least one animal in each cohort had parasite counts greater than zero. In short, every animal in our dataset were exposed to natural infestation of the three different evaluated parasites.

2.2.2 Genetic parameters for body weight and host resilience

We used single trait linear random regression models (STM) where BW in each ME was considered as a dependent variable (trait) and the median counts of parasites as an independent variable. It is important to emphasize that in this study the random slope coefficient was considered as the HR to parasite. The analysis was performed for each ME and each parasite separately, and no effect of co-infection was considered. The STM was performed using Bayesian inference methodology, and can be described as:
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\[ y_{ijkl} = C_j + d_i M_{(k)} + b_0 + b_1 X_{(i)} + a_{0_{i}} + a_{1_{i}} X_{(j)} + e_{ijkl}, \]

where \( y_{ijkl} \) represents the weight of the animal \( i \), evaluated at the cohort \( j \), with the age \( k \) and submitted to an environmental parasite burden \( l \); \( C_j \), is the systematic effect of the cohort; \( d_i \), is the slope to fit the effect of age in which each animal was evaluated; \( M_{(k)} \) is the age (in days) of the animals at the day of evaluation; \( b_0 \) and \( b_1 \) are the intercept and slope to fit the BW mean trajectory along the parasite burden, respectively; \( X_{(j)} \), represents the median of parasite counts (TICK or GIN or EIM) of the animals’ cohort; \( a_{0_{i}} \) and \( a_{1_{i}} \), represent the random intercept and slope to fit the additive genetic effect of each animal \( i \), respectively; and \( e_{ijkl} \), represents the error associated with each observation.

For this model, we assumed that intercept and slope were associated, in such a way that the covariance matrix for the random coefficients of the model (G0) can be described as:

\[
G_0 = \begin{bmatrix}
\sigma^2_{\text{int}} & \sigma_{\text{int,slope}} \\
\sigma_{\text{int,slope}} & \sigma^2_{\text{slope}}
\end{bmatrix};
\]

where \( \sigma^2_{\text{int}} \) and \( \sigma^2_{\text{slope}} \) are the additive genetic variances for the intercept and slope that adjusted parasite burden for each trait, respectively; and \( \sigma_{\text{int,slope}} \), is the additive genetic covariance between intercept and slopes for a same trait.

The additive genetic variances for body weight in each observed parasite burden were estimated by the product \( P \otimes G_0 \otimes P' \). The \( P \) matrix has the number of lines equal to the number of different environments and two columns. The first column of \( P \) is a vector of 1 for adjusting the intercept, and the second column is the vector containing the observed parasite burden; \( P' \) is the transpose of \( P \) matrix; the matrix \( G_0 \) is the covariance matrix between the regression coefficients, and; \( \otimes \) is the direct product operator. Further information about the STM are presented at the Supplementary Material and Methods.

2.2.3 Genome-wide association studies

Genome-wide Association Studies (GWAS) were carried out for HR to the three different parasites and BW measured in each ME. To perform GWAS we used the SNP & Variation Suite v8.8.3 software [21]. A mixed model was used to estimate the solutions for each SNP marker that passed quality control. As a result, we report on the association test \( P\)-values for each SNP and each studied trait (BW, and HR to TICK, GIN, and EIM), in each ME. The GWAS model used for BW can be described as:

\[ BW = Xb + Zu + s + e; \]

where \( BW \) is the vector with body weight information of each animal in each evaluated age; \( X \) is the incidence matrix for the fixed covariates (cohort and age); \( b \) is the vector of solutions for the fixed effects; \( Z \) is a incidence matrix for the genetic additive random effects (estimated from polymorphisms); \( u \), is the vector with solutions for the random additive genetic effects related to the observations; \( s \), represents the SNP effects vector; and \( e \), represents the error associated with each observation. SNP & Variation Suite v8.8.3 [21] uses a restricted maximum likelihood to estimate the solutions for the unknown parameters of the model.
The breeding values (EBV) estimated for HR to each parasite in each ME using STM were considered as the phenotypes of HR for GWAS analysis. No fixed effect was considered since all the known environmental effects were considered in the previous analysis to estimate the breeding values for HR. The model used for the GWAS analysis for HR can be described as:

\[ HR = Zu + s + e; \]

Where HR is the vector of EBVs for host resilience to TICK, GIN, or EIM in each ME and the other terms as previously described.

In the GWAS mixed models, for BW and HR, we used a genomic relationship matrix (GRM) [22]. The GRM was built from our SNP Panel with 21,667 markers and their genotypes for the 1,075 animals available after quality control. The GRM was generated using the SNP & Variation Suite v8.8.3 [21], and a full dosage compensation correction was applied to include the X chromosome markers in the estimate for the GRM. This software uses the algorithm proposed by Taylor [23]. The effect of sex over the solutions for SNPs located in the X chromosome was also considered in this GWAS analysis.

The heritability for BW and HR were estimated based on the variances obtained from the markers (using GRM), so we refer to that estimate as "SNP-derived heritability". It is important to highlight that, in the present study, the heritability for BW was estimated based in both GRM and the pedigree-based relationship matrix, so for BW we presented both the conventional heritability and the SNP-derived heritability estimates.

The correlation matrix among BW and HR to TICK, GIN, and EIM measured in each ME was computed by the Pearson correlations between the SNP effects (SNP correlations) for each one of the traits, as proposed by Fortes et al. [24]. To compute the SNP correlation matrix, we first standardized the SNP effects estimates by its standard error.

2.2.4 Genetic variances for host resilience across ages

We used two-trait linear random regression models (TTM) to model BW in each ME in function of an intercept and a slope defined by the median counts of parasites. The ME information (ME.331, ME.385, ME.443, ME.498, and ME.555) were analyzed by two-trait analysis, to achieve convergence of the parameters of the model. The TTM can be described by the same equation of STM. For this model, the covariance matrix for the random effects (G1) was assumed as:

\[
G1 = \begin{bmatrix}
\sigma_{int_1}^2 & \sigma_{int_1,slope_1} & \sigma_{int_1,int_2} & 0 \\
\sigma_{slope_1}^2 & 0 & \sigma_{slope_1,slope_2} & \sigma_{slope_1,slope_2} \\
\sigma_{int_2}^2 & \sigma_{int_2, slope_2} & \sigma_{slope_2}^2 & 0 \\
\text{sym} & \text{sym} & \text{sym} & \text{sym}
\end{bmatrix};
\]

where \( \sigma_{int_1}^2 \) and \( \sigma_{slope_1}^2 \) are the additive genetic variances for the intercept and slope that adjusted parasite burden for each BW, respectively; \( \sigma_{int_1,slope_1} \), is the additive genetic covariance between intercept and slope for a same trait; \( \sigma_{int_1,int_2} \), is the additive genetic covariance between the intercepts of the two evaluated BW; and \( \sigma_{slope_1,slope_2} \), is the additive genetic covariance between the slopes of the two evaluated BW.
Detailed information about the TTM and its assumptions can be found in the Supplementary Materials and Methods. The genetic correlations between the HR for each parasite at different ages were used to infer about the genetic association of HR across the studied growth trajectory. The genetic correlations estimated by TTM and the SNP correlations were used to study if similar mechanisms could explain the host resilience to TICK, GIN, and EIM.

2.2.5 Quantitative trait locus associated with host resilience

Statistical meta-analysis combining the results of the GWAS performed for HR to each parasite in the five different ME were performed to identify genomic regions associated to HR to TICK, GIN and EIM separately. This approach was used to identify regions that are associated to HR to each parasite with more certainty, and despite age. The Sample-Size-Based approach described by Willer et al. [25] was used to perform the meta-analysis using the SNP & Variation Suite v8.8.3 [21]. In summary, from the P-values, effect direction, and sample size of each GWAS, a Z-score and an overall P-value for each marker were calculated. The meta-analysis was performed for markers that had valid solutions in at least 2 studies and no genomic control was performed during the meta-analyses. Afterwards, we used a Bonferroni correction for multiple testing to define the threshold for SNP effect significance. Only SNPs with P-values < $2.31 \times 10^{-6}$ were considered as significant. Another threshold of P-values < $10^{-4}$ was used to infer suggestive SNPs, a common practice in GWAS [26,27].

Based on meta-analysis results, quantitative trait locus (QTL) associated with each trait were described. The QTL boundaries were defined as follow: First, for each bovine chromosomes (CHR), an initial peak SNP was defined as the SNP with the lowest P-value; Second, around the peak SNP, regions of 0.5Mbp up and downstream were searched for other significant SNPs. If inside this interval we identified other significative SNP, the boundaries of the QTL were expanded to include the SNP and another 0.5Mbp (up and downstream) was investigated. The process was repeated until there was no more significative SNPs in these 0.5Mbp windows. Finally, a new peak SNP was called if there was a significative SNP in the same CHR but outside of the boundaries of the first QTL. The process was repeated within each CHR until no more peak SNPs could be identified.

To provide additional evidence to QTLs associated to HT, we imposed one more criterion. Only regions with at least four significative or suggestive SNPs were considered as a QTL (adapted from van den Berg et al., 2016). Suggestive SNPs had a P-value < $10^{-4}$ and to be considered as supporting evidence for the QTL they had to satisfy one more condition: to have above average LD with the peak or other significant SNP in the QTL. The LD between SNPs was evaluated by the D prime ($D'$) estimated using the expectation-maximization method at pairwise analysis carried with the SNP & Variation Suite v8.8.3 [21]. SNPs were considered in high LD when $D'$ was greater than the mean + 2 standard deviations of the $D'$ computed between all combinations of markers for each CHR. For the traits in which no QTLs could be described, we identified the genes around isolated significant SNPs (0.5Mbp downstream and upstream the SNP position).

We looked for genes located inside the QTL boundaries using the ARS-UCD1.2 bovine genome assembly (available at www.ncbi.nlm.nih.gov/assembly/GCA_0002263795.2). The search for genes was made using the GALLO package [29] of R software [30]. Genes located inside QTLs or around isolated significant SNPs were considered as candidate genes. Candidate genes formed target gene lists that were confronted with a trained gene list in functional analysis for candidate gene prioritization, as described below.

The trained list of genes was constructed using keywords (Table S2) that described each of evaluated phenotypes (BW and HR to the three parasites). These lists were built on GUILDify v2.0 [31], which
is a web application for phenotypic characterization of genes. GUILDify searches for genes starting from user-provided keywords in the Biologic Interaction and Network Analysis (BIANA) knowledge database. These genes associated with the keywords are used as seeds to generate the protein interaction networks, for the selected organism, analyzed with graph theory algorithms to prioritize new disease genes [31]. In the present study, the selected model organism was *Homo sapiens*, since bovine was not an option. The Netscore prioritization algorithm from the GUILD package was used (with repetition = 3 and interaction = 2; default values of GUILDify). The output of GUILDify is a trained list of genes, ranked according to the interaction network. The first 100 genes were used as the trained gene list for each studied trait.

Candidate gene prioritization analysis were conducted with ToppGene Suite [32]. These analyses were performed in two-steps. First, for each trait, a functional enrichment analysis was performed to verify if the trained gene list was enriched for any functional category or parameter. We used Gene Ontology (Molecular function, Biological process, and Cellular component), Human phenotype, Mouse phenotype, Pathway, PubMed, Transcription factor binding site, Co-expression, and Disease as training databases to identify over-representative terms from the trained gene list. The *P*-value cut-off for each training parameter was 0.05 with a False Discovery Rate correction. After this step a representative profile of the trained gene list was obtained.

In the second step a similarity score was generated for each gene in our candidate gene lists. This score is created by functional annotation of the candidate gene followed by a comparison of its function to each enriched term, learned in the training step. The similarity score calculation and the *P*-values associated to them are described in Chen et al. [32]. In summary, a fuzzy-based similarity measure is applied for categorical terms [33], and Pearson correlation between the test gene and the enriched gene lists is applied for quantitative functional parameters. In the case of a missing value (for instance, lack of one or more annotations for a test gene), the score is set to −1. Otherwise, it is a real value in [0, 1] [32].

The overall scores and *P*-values are obtained with a meta-analyses that considers all the functional categories annotated [32]. The prioritized genes were considered those with an overall *P*-value ≤ 0.05. For the candidate gene prioritization analysis, we used the default setting in ToppGene Suite that is a background gene set from the genome for computing the *P*-value with 5000 coding genes and two features to be considered for prioritization.

3 Results

3.1 Environmental parasite burden

The environmental parasite loads observed here were low (Table 1). It might be a consequence of preventive treatment applied to 65% of animals randomly chosen on the first day of each performance test. A common strategy to estimate the total count of parasite per animal is to multiply by 2 the number of engorged females observed in the right side of each animals and it is important to highlight that the parasite counts reported here were not multiplied by any constant. For instance, ticks’ count represents the number of ticks observed on the right side of the animals, and the eggs and oocysts’ counts represent the number of parasites observed on the McMaster chamber (Table 1).

3.2 Genetic parameters for body weight and genotype x parasite burden interaction

In general, there is no significant difference between genetic parameters of intercept and slope coefficients estimated by STM (Table 2) and TTM (Table S3). Except for genetic additive variances
of intercept coefficient at ME.555 when EIM parasite burden was considered in the model. In this case, the variance (and high posterior density intervals with 90% of samples - HPD90) of the intercept of 192.4 (94.15; 292.5) estimated by STM (Table 2) was significantly higher than the value of 40.30 (1.42; 90.39) estimated by TTM (Table S3). Therefore, the results of STM are presented and discussed in the main text (Table 2, Figure 2) and the results for TTM are presented on the supplementary Table S3 and Supplementary Figure 2.

The SNP-derived heritability (average ± standard error) of BW (Table 3) at each ME varied from low (0.09±0.06 at ME.331) to moderate magnitude (0.23±0.06 at ME.555), showing that genetic improvement of BW can be achieved through selection. Those values were similar to the heritability of BW estimated by STM (Figure 2) and TTM (Supplementary Figure 2).

There was no difference among genetic parameters of BW when TICK, GIN, or EIM burden were used as an environmental gradient (Figure 2 and Supplementary Figure 2), which is expected since the genetic parameters for BW measured in the same population should not differ in function of the statistical model used to estimate them. Moreover, large HPD90 were related to the posterior means of additive variance and heritability of BW in each ME, showing that there is no impact of age, from 331 to 555 days old, on genetic parameters of BW (Figure 2 and Supplementary Figure 2).

A rising trend for additive variance and heritability of BW was observed across TICK, GIN, and EIM burden trajectory (Figure 2 and Supplementary Figure 2). For instance, the posterior mean for heritability of BW varied from 0.09 to 0.44 at ME.331, from 0.13 to 0.51 at ME.385, from 0.13 to 0.54 at ME.443, from 0.16 to 0.45 at ME.498 and from 0.11 to 0.42 at ME.555. Despite the difference between heritability estimates for BW when parasite count was zero and maximum (16 for TICK, 11 for GIN and 10.5 for EIM), the HPD90 related to those posterior means were large showing no significant differences between them (Figure 2).

The SNP correlations between the BW measured in each ME are high, with averages (standard errors) ranging from 0.705 (0.005) between BW at ME.331 and BW at ME.555 to 0.882 (0.003) between BW at ME.498 and BW at ME.555 (Figure 3). These high SNP correlations between BW measured from 331 to 555 days old are in accordance with GWAS results for BW in which markers located at chromosome 6 and 14 were suggestively associated with BW measured at all evaluated ME (Supplementary Figure 3). Also, BW measured at different ages could be considered repeated measures of the same phenotype, instead of being perceived as independent traits since animals that are heavier in the beginning of performance tests tend to be heavier in the end.

The genetic correlations of BW measured at each ME between different parasite burden varied. They ranged from high and positive, between animals submitted to similar parasite burden, to moderate and negative, between animals submitted to extreme different parasite burden (Figures 4, 5, 6), demonstrating the effect of parasitic burden on BW genetic parameters. For instance, the smallest estimates of genetic correlation for BW were obtained between zero and maximum parasite counts (maximum count of 16 for TICK, 11 for GIN and 10.5 for EIM), with a posteriori means (HPD90) of -0.29 (-1.00; 0.37) at ME.331 for TICK (Figure 4); -0.19 (-0.69; 0.31) at ME.385 for GIN (Figure 5); and -0.46 (-0.90; -0.02) at ME.385 for EIM (Figure 6). Thus, demonstrating the effect of parasitic burden on BW genetic parameters.

It is possible to verify that negative correlations between BW measured at zero and maximum counts occurred at the ME in which negative and significant covariances were estimated between intercept and slope (Table 2). The genetic correlations between intercept and slope (Table 2, Table S3) and the
correlations between BW measurements at each ME across varying parasite burden conditions (Figures 4, 5, and 6) indicate that animals’ genetic performance (that depend on animal’s genotype) might differ according to the parasite burden to which they are submitted to, which means that some level of genotype x environmental interactions that influence BW under natural infestation conditions can be verified. For breeding purposes, the identification of this interaction might help to define the environmental conditions and management practices to which candidate animals will be submitted.

3.3 Genetic parameters for host resilience and their association with body weight

In this study, HR was estimated as a genetic component of BW, the slope of random regression models. Therefore, genetic variance estimates were obtained for HR, but heritability was not estimated. There was no impact of age of measurement on HR genetic variances to TICK, GIN, or EIM regardless of the model used (STM and TTM) since HPD90 of genetic variance for HR across ME overlapped (Table 2, Table S3). Also, when we compare STM and TTM results, for the same ME, the HPD90 of HR genetic variance to TICK, GIN or EIM overlapped again. These overlaps are evidence for the fact that the HR’s genetic variances were similar across age groups and statistical models.

The SNP-derived heritabilities for HR to TICK, GIN, and EIM at each ME were computed through GWAS analyses when the slopes solutions were considered as HR phenotype. As expected, these estimates presented high magnitude (Table 3), ranging from 0.76 to 0.87 for HR to TICK, from 0.80 to 0.93 for HR to GIN, and from 0.77 to 0.84 for HR to EIM.

The genetic association between BW and HR was measured throughout SNP correlations between the traits (Figure 3). In our study, both positive and zero correlations were considered as favourable. Negative and unfavourable correlations were observed between BW and HR to TICK at ME.331 (-0.648±0.005), ME.443 (-0.307±0.006), and ME.498 (-0.148±0.007), between BW and HR to GIN at ME.385 (-0.038±0.007), and between BW and HR to EIM at ME.555 (-0.081±0.007 – Figure 3).

3.4 Host resilience to parasitic burden at five ages are independent traits

The posterior means of genetic variances for HR to TICK, GIN, and EIM were similar in each ME (Table 2, Table S3) since the HPD90 overlapped. The genetic correlations between HR measured from 331 to 555 days old ranged from 0.13 (ME.331 x ME.555) to 0.40 (ME.443 x ME.498) for HR to TICK; from 0.11 (ME.385 x ME.498) to 0.52 (ME.385 x ME.443) for HR to GIN; and from 0.25 (ME.385 x ME.443) to 0.56 (ME.498 x ME.555) for HR to EIM (Figure 7). The genetic correlations previously described for HR to TICK, 0.13 at ME.331 x ME.555 and GIN, 0.11 at ME.385 x ME.498 did not differ from zero, since HPD90 includes zero value.

Furthermore, the SNP correlations between HR to each parasite measured from 331 to 555 days old showed low to moderate magnitude (Figure 3). SNP correlation between HR to TICK measured from 331 to 555 days old ranged from -0.364 ± 0.006 (ME.331 x ME.555) to 0.296 ± 0.006 (ME.385 x ME.555); from -0.101 ± 0.007 (ME.331 x ME.498) to 0.505 ± 0.006 (ME.331 x ME.385) for HR to GIN measured from 331 to 555 days old; and from 0.368 ± 0.006 (ME.498 x ME.555) to 0.794 ± 0.004 (ME.443 x ME.498) for HR to EIM measured from 331 to 555 days old. Moreover, the genetic correlations between HR of a same parasite at different ages and SNP correlations were in accordance with GWAS results where suggestively associated markers for TICK, GIN, or EIM were age-specific (Supplementary Figure 4, Supplementary Figure 5, and Supplementary Figure 6, respectively).

Therefore, the trend of HR to parasites changes at different ages, for instance HR should be considered as an independent trait.
3.5 Candidate genes and pathways associated with host resilience to TICK, GIN, and EIM

The search for genes associated with HR was carried only at the regions defined as QTL or in the vicinity of significant SNP, considering only the meta-analysis results, and not the results for each age separately (Figure 8). The aim was to identify candidate genes that might explain the genetic correlations observed between HR to each parasite across the different ages. In short, we focused on the associations (and therefore candidate genes) that had stronger evidence by combining in one analysis all the HR data available for each parasite.

There were no relevant QTLs identified for HR to TICK through the meta-analysis. However, we identified candidate genes nearby significant SNPs. A total of 52 genes formed the candidate list for HR to TICK: 11 on CHR 2, 6 on CHR16, and 35 on CHR19. (Table 4). Out of these candidates, 21 were prioritized in our candidate gene prioritization analyses. Information about genes prioritized for HR to TICK is presented at Table S4.

There were significant SNPs associated to HR to GIN on CHR 9, CHR 14 and CHR 28 (Table 5), but no QTLs were defined. Moreover, 37 genes associated to HR to GIN were identified around peak SNPs, 13 at CHR 9, 16 at CHR 14 (8 around each peak SNP), and 8 at CHR 28. Out of these 3, 5 (3 and 2, around each peak SNP on CHR 14), and 2 genes located at CHR 9, 16 and 14, respectively, were prioritized. Information about genes prioritized for HR to GIN is presented at Table S5.

A total of 137 significant SNPs distributed across CHRs, except CHR 25, were associated with HR to EIM. We identified 5 QTLs located at CHRs 4, 6, 7, 12, 13 associated with HR to EIM through meta-analysis (Table 6). Information about number of SNPs and linkage disequilibrium thresholds used to define the QTL boundaries are presented at Table 7. A total of 47 genes were located inside these QTLs (Table 6). From these, 16 genes were prioritized. Information about genes prioritized analyses for HR to EIM is presented at Table S6.

4 Discussion

4.1 Environmental parasite burden

The median counts of parasites were used as an environmental gradient since the environmental load is highly dependent of how infested the animals are. Regarding different parasites, there is no direct transmission from one animal to another, but the animals raised in the same environment contaminate the pasture and allow the parasite to complete its life cycle. For instance, cattle ticks have multiple stages of development – egg, larva, nymph, and adult. Tick’s adult females realize the oviposition on the pasture, where hatching happens. Larvae, nymphs, and adults parasitize the host for feeding, and then drop off on the pasture again to continue its development [34]. Gastrointestinal parasites also have multiple stages of development. In general, adult females of gastrointestinal nematodes parasitize the abomasum and the intestines and produce eggs, which are eliminated together with the fecal mass. Egg’s hatching and larvae molts to reach the infective third larval stage happen inside of the faecal material and the infective larva goes to the forage where they are ingested by the animals [19].

Regarding *Eimeria* spp., oocysts are shed with the faeces. Under proper environmental conditions the oocyst develops to form a sporulated oocyst, that is infective to other cattle. After ingestion, the oocysts release sporozoites in the intestine where the endogenous phase of the life cycle happen [3].

The low parasite load observed here might be partially explained by the adoption of rotational grazing [35], and the use of prophylactic parasite control strategy. And it is a common practice on cattle farms worldwide. According to World Organization for Animal Heath from 2013 to 2016 18 of the 18
countries in America, 27 of the 28 countries in Africa, 15 of the 17 countries in Asia and the Pacific, and 40 of the 40 European countries reported the use of antimicrobial agents in cattle production [36]. Moreover, Cruvinel et al. [37] evaluated the prevalence of agents causing diarrhea in dairy cattle raised in 872 different farms distributed across eight different states in Brazil (which represent 80% of Brazil’s total milk yield production). They concluded that only 195 farms (22%) do not use any prophylactic drug to control parasites load, including *Eimeria* spp. and gastrointestinal nematodes. Furthermore, more than one-half of beef cattle farmers in USA usually deworm different categories of animals one or more times per year [38]. Therefore, environment with controlled parasite burden through prophylactic treatment represents the reality of commercial farms.

Even though under low parasite load challenge we could observe the impact of parasite burden on the body weight, and a raising trend for the heritability of body weight as the loads increased. It is expected that more challenging environments, this means, higher parasite loads, can lead to more significant effects parasite burden on both BW and genetic parameters estimates for HR (Falconer, 1990). Considering this, the low parasite loads observed on the present study might be a limiting aspect of our study for the study of the genetic architecture of HR to different parasites, and further genomic regions associated with these traits could be found when applying the same methodology we used on a population submitted to higher burdens.

The observed median counts reported here are similar of other data sets available on the literature. For instance, Martins et al. [18] performed repeated tick counts on 11 Brangus and 12 Nellore growing bulls raised on a commercial farm located at Mato Grosso do Sul – Brazil. The animals were naturally infested and no prophylactic treatment was performed [18]. The authors observed 45.51 ± 20.91 and 10.08 ± 2.00 (mean ± standard deviation) ticks on the Brangus and Nellore animals, respectively, and this numbers represent the tick count on the entire animal (both left and right sides). The average of 17.4±24.6 ticks per animal were verified across 1332 animals of four different Colombian *Bos taurus* cattle breeds, raised on different regions of the country [40]. More than 50% of the evaluated animals had between 0-10 ticks on their body. These animals were naturally infested, and no parasite control methods were carried out during the experimental period [40]. Regarding gastrointestinal parasites the average of 11.35 ± 22.57 eggs per gram of faeces were observed from a population of 1166 German Black and White dairy cows, pasture raised, and naturally infected with GIN [41]. In short, we would like to highlight that although the low parasite burdens and the possible impact of prophylactic measures taken in the studied farm, the summary statistics of the dataset we presented here is similar to other studies that might characterize a commercial farm environment and should be used to evaluate the genetic parameters of health traits in beef cattle industry.

4.2 Genetic parameters for body weight and genotype x parasite burden interaction

The similarity between BW heritabilities estimated with genomics (SNP-derived heritability), STM, and TTM serve as evidence for the adequacy of the low-density SNP panel (27K – Z-chip V2, Neogen, Lincoln, Nebraska, EUA) to capture the polygenetic component of the additive variance observed for BW in Nellore cattle. The heritability estimates of BW in this population were lower than the estimates found on the literature for Nellore cattle of similar ages raised in Brazil, using pedigree information only, and single and multiple trait models [42], and similar to the heritability estimated for BW at 555 days old for the same population [43]. It is important to note that selection can lead to lower genetic variability of a trait, and consequently, lower heritability [44]. Therefore, years of selection to BW practiced since 1978 by a genetic nucleus farm with non-inclusion of external candidates such as Mundo Novo might explain the lower heritability estimates obtained here. It is important to highlight that those two genetic management practices, selection and non-inclusion of external candidates,
promoted significant benefits to this population that is highly regarded as a genetic disseminator of the
Nellore breed in Brazil.

In the present study we did not find significant changes in BW heritability estimates across varying
gradients of parasite burden. The natural infestations and overall low parasite burden observed in this
data might partially explain the constant heritability, because challenging environments might lead to
increases in heritability estimates [45,46]. For instance, heritability for FAMACHA score in ram and
ewe lambs submitted to high worms burden, mostly *Haemonchus contortus*, *Trichostrongylus* spp.,
and *Teladorsagia* spp., were significantly higher than the heritability obtained in low and moderate
parasite burden (Riley and Van Wyk, 2009). FAMACHA score is a common veterinarian approach to
evaluate the parasite burden, based on the colour of the animals’ eye mucosa (indicative of anaemia).
Moreover, an uprise trend in heritability estimates for milk yield was observed with increased
temperature-humidity index, a direct indicator of heat stress (Lee et al., 2011). Therefore, we expect
that BW evaluation on infested environments, with higher range parasite counts than those observed
here, might lead to variation in heritability estimates of BW across varying gradients of parasite burden.

We were able to verify a rising trend on heritability means (mainly for HR to TICK and GIN at ME.555,
and HR to EIM at all ME) however these values were followed by large HPD. It is important to
highlight that Mundo Novo farm has an efficient animal husbandry program, including strategic
parasite control, that correspond to the outstanding practices of a nucleus farm and it explains the low
parasite burden observed in our study, including the more uniform weight gain.

Our results showed low genetic correlations between BW measured at the lowest and the highest
parasite loads specially at younger ages (331 days old compared to 555 days old), indicating potential
genotype by parasite burden interaction when animals are raised in very low parasite burden (cohort’s
median count equal zero) and infested environments. Hollema et al. [47] also emphasize the importance
of considering the genotype x worm burden interaction for growth rate in Australian Merino sheep to
increase the efficiency of selection for animals that are more parasite resistant and more resilient to
environmental worm challenge. However, these authors verified significant decrease on the heritability
for growth rate with the increase of worm burden [47]. It becomes even more important for growth
selection on pastured systems in tropical areas that are typically under different levels of natural
infestation, and that apply different strategies for parasite control.

4.3 Genetic parameters for host resilience and their association with body weight

The SNP-derived heritability estimated for HS in the present study are high, but they do not indicate
that HS is highly heritable. In fact, these values are a statistical artefact since the phenotype of HS is
itself an estimated breeding value. However, the SNP-derived heritability indicate that breeding values
estimated using pedigree-based genetic evaluations can be efficiently explained by the genomic
similarity between individuals. There is genetic variance for HR to parasites and genetic improvement
for this trait can be achieved through selection, even though the genetic gain across generation might
be slow. The SNP correlations between HR and BW were obtained through standardized values, where
the SNP effects were divided by its standard error. Standardized values reduced the impact of large
differences on genetic variance of BW and HR obtained here when computing SNP correlations
estimates. Furthermore, the SNP correlations were particularly interesting since the effect of parasite
infestation was not considered in the BW’s GWAS analyses. In this sense, while genetic correlation
between intercept and HR indicates the presence of genotype x parasite burden interaction for BW, the
SNP correlations indicates some genetic association between BW and HR to parasites.
Unfavourable correlations were observed mainly between BW and HR to TICK at ME.331, ME.443, and ME.498. These correlations were moderate and negative at ME.331 but were reducing in magnitude as animals aged. Two aspects must be discussed here. First, the fact that inside each age category (ME), heavier animals are expected to be larger in size, and have a wider skin surface with a denser vasculature, which was already suggested as a possible explanation for the associations between parasite burden and BW [40], and might also explain the association between BW and HR. Second, the immune response mechanisms might vary with age [48–51] and so younger animals (for instance those evaluated in ME.331) might differ from older ones (like those evaluated in ME.555) in terms of HR. Altogether, these two aspects can justify the differences observed in the SNP correlations between HR to TICK and BW in the different ME. Further discussion about the age effect on HR expression is presented below.

4.4 Host resilience to parasitic burden at five ages are independent traits

The low to moderate magnitude of genetic correlation between slopes at each ME in TTM and SNP correlations for HR to parasites in each ME indicate that HR to TICK, GIN or EIM measured at different ages are independent traits, showing no benefit of using indirect selection to improve HR in older animals through selection of younger ones and vice versa. It is already known that the severity of infectious diseases can vary dramatically across ages, possibly due to the immaturity of the immune system of young animals [52]. Similarly, it is possible that host resilience also changes across ages, depending on the exposure to co-infection and different parasites in each growing phase [53].

4.5 Candidate genes and pathways associated with host resilience

4.5.1 Candidate genes and pathways associated with host resilience to TICK

We found here a significative association between the genes WNT4 and CFH and HR to TICK. The WNT4 gene was recently associated with the suppression of type 2 immunity (Hung et al., 2019), which assists with the resolution of cell-mediated inflammation [55]. The CFH gene was associated with risks to diseases in which the etiology is related to complement dysregulation [56]. This gene’s transcript is the complement factor H, which contributes to the regulation of complement activation and assists in modulating the response on host cell surfaces [56,57]. In short, WNT4 and CFH are known for their immune function and our results suggest they are linked to HR to TICK. Inhibiting the complex mechanisms of host homeostasis, including the immune system, contributes to successful tick blood feeding [58]. Future research may focus on how these prioritized genes may interfere with tick infestations.

Another homeostasis-related mechanism that appears to be relevant to the expression of HR to TICK is apoptosis, suggested by the association of YWHAE and SCARF1 genes. Apoptosis is the mechanisms that ensures that damaged, aged, or excess cells are deleted in a regulated manner that is not harmful to the host and plays an essential role in the development and maintenance of all mammalian tissues [59]. The insertion of the tick’s mouthparts during tick blood feeding leads to damages of epidermis and dermis cells [60]. In response to damage, pathogen-associated and or damage-associated molecular patterns are detected, and leukocytes aggregate near to the site of lesion [61]. After eliminating the initial threat, leukocyte recruitment ceases, and the previously recruited cells are disposed, mainly mediated by apoptotic cells that are subsequently phagocyted [61]. A rapid and immunologically ‘clean’ removal of apoptotic cells by neighbouring phagocytic cells is essential for the maintenance of homeostasis and avoidance of inflammation [62]. This mechanism may explain why genes that are relevant to apoptosis, like YWHAE, and SCARF1, may also be important for HR to TICK.
YWHAE is a protein isoform of 14-3-3 family of eukaryotic proteins which have anti-apoptotic activity, and regulate members of the mitochondrial apoptotic machinery, as well as a staggering number of signalling molecules that mediate the transmission of survival and death signals to the mitochondrial death machinery [63]. SCARF1 is one of the main genes mediating the clearance of apoptotic cells [64]. These mechanisms are part of a delicate balance in order to maintain the homeostasis. To understand how they take part on the expression of HR further studies are necessary. It would be useful, for example, to evaluate if these genes are up or downregulated in more resilient hosts upon infestation.

Alpha-2 pigment epithelium derived factors secretion (that are transcripts of SERPINF1 and SERPINF2 genes) were related to HR to TICK. SERPINF1 was associated with mice full-thickness cutaneous wound healing by promoting epithelial basal cell and hair follicle stem cell proliferation. SERPINF2 acts on other aspects of the wound healing, in the clearance of the anticoagulant plasma protein C, inhibiting its actions and enhancing the coagulation process [65,66]. Altogether, the mechanisms controlled by SERPINF1 and SERPINF2 might indicate the relevance of fast wound healing on the expression of HR to TICK.

The direct effect of tick saliva might also activate specific mechanisms of HR. Salp15 is the first protein associated with the immunosuppressive activity of *Ixodes scapularis* tick saliva (Anguita et al., 2002). CDC42 activation can act as a stimulus to F-actin polymerization, and the amount of F-actin was reduced upon pretreatment of T cells with Salp15 in mice [67]. The complexity of events at the tick host interface is increased by the process in which injection of saliva occurs alternatingly with uptake of blood as well as of digested tissues at an increasing rate over the course of blood feeding [58]. It was demonstrated that Salp15 inhibited the activation of CD4+ T-cells in mammalian hosts, resulting in decreased activation of the transcription factor NF-κB [68].

4.5.2 Candidate genes and pathways associated with host resilience to GIN

In ruminants, third stage larvae of GIN exsheath in the rumen and further development takes place in the mucosa of the abomasum or the intestine [5]. Parasite antigens are presented to T lymphocytes and after, the T cells further regulate the host response against the GIN [69]. In geographical areas in which nematode parasites are endemic, immunity to infection in previously exposed individuals is associated with expression of T-helper type-2 (TH2) cytokines and an inverse association between TH2 cytokines and susceptibility to GIN in younger children (between 4 and 13 years old) was verified [70].

AGO2 was associated in the present study with HR to GIN and previously was associated with host response *Toxoplasma gondii* [71], *Cryptosporidium parvum* [72], and *Plasmodium falciparum* (Mantel et al., 2016). The Argonaute (AGO) proteins are key components of miRNA-induced silencing complex (miRISC) that bind miRNA and direct the miRNA to its target mRNAand regulates TLR signaling [74,75]. Cellular miRNAs are released from cells both membrane free or inside exosomes, which are extracellular micro vesicles that carry bio reactive macromolecules such as nucleic acids, proteins, and lipids, and therefore may contribute to the pathogenesis of disease [76].

The host’ gastrointestinal infection by *Salmonella enterica* serovar Typhimurium [77] and intestinal autoimmune diseases as Chron’s disease [75] have also been associated with posttranscriptional regulation of immune response by miRNA machinery (pathway in which AGO2 plays important role). The use of AGO2-miRNA pathway in the regulation of immune response of cattle infected by GIN was not described yet, however previous results presented here as well as the significant association of AGO2 with HR to GIN might indicate the relevance of this pathway.
The restitution of intestinal epithelial barrier damage, that might be caused by GIN infections, is another mechanism that appears to be relevant for the expression of HR. CXCL12 activates the chemokine receptor CXCR4 and enhances intestinal epithelial wound healing through reorganization of the actin cytoskeleton [78]. CXCL12 is a constitutive and inflammatory chemokine in the intestinal immune system, expressed by normal intestinal epithelial cells [79]. It was up-regulated in both catfish intestine submitted to following experimental infection of Edwardsiella ictaluri, the causative bacterium of enteric septicemia of catfish [80], and human intestine of patients with inflammatory bowel disease [79]. No associations of CXCL12 expression in the intestine of animals submitted to gastrointestinal nematodes burden was published yet. However, our results indicate that this might be an important candidate gene for the study of HR to GIN.

4.5.3 Candidate genes and pathways associated with host resilience to EIM

The chemokines also play an important role on the development of HR to EIM, as the association of genes CXCL9, CXCL10, and CXCL11 suggests. These genes transcripts are proinflammatory chemokines that are released from the intestinal epithelium [81]. Increased levels of both CXCL9 and CLCX11 transcripts in the gut tissue of susceptible mice artificially infected with Trichuris muris, an intestinal nematode parasite, were identified [82]. Oppositely, the up-regulation of these genes were not verified in resistant mice subjected to the same artificial infestation. Furthermore, in vivo neutralization of CXCL10 in infected susceptible mice caused a significant reduction in worm burden and the treated group of animals showed a highly significant increase in the rate of epithelial cell turnover when compared with untreated animals [83]. Furthermore, Cliffe et al. [83] demonstrated that CXCL10 had no effect on the ongoing TH1 immune response (that is a characteristic of a susceptible animal), which strongly suggests that epithelial cell turnover alone can mediate worm expulsion.

Also, Reid-Yu et al. [84] demonstrated that CXCL9 plays an important role in antimicrobial defense in the infected and inflamed gut of mice artificially infected by the bacteria Citrobacter rodentium. This activity, independent of the chemokine receptor CXCR3 or an adaptive immune response, protects the gut from crypt invasion by C. rodentium and the tissue damage that ensues [84]. Although Eimeria spp. is a protozoan, which have life cycles that differ from worms or bacteria, the association of CXCL9, CXCL10, and CXCL11 indicates that immune responses mediated by chemokines is probably an important mechanism for HR to protozoan that parasite the intestine as well, represented by EIM in the present study.

IRS2, LIG4, and TNFSF13B are modulators of antibody levels that were previously associated with human susceptibility to Ascaris lumbricoides infection, an endemic disease at tropical areas and caused by nematodes [85]. TNFSF13B (also known as BAFF) production by intestinal epithelial cells is stimulated by the commensal bacteria present in intestine, and plays important role on the maturation of naïve B cells into mature cells with a process called class-switch recombination [86]. This is the process by which proliferating B cells rearrange their DNA to switch from expressing IgM (or another class of immunoglobulin) to expressing a different immunoglobulin heavy-chain constant region, thereby producing antibody with different effector functions [86].

We did not find in literature studies that described the relevance of the genes presented here in the development of coccidiosis (diseases caused by coccidian protozoan, as Eimeria spp.). However, Kim et al. [87] speculated that the expression of TNFSF13B verified in the intestinal tissue of chicken orally infected with Eimeria acervulina may have increased upon coccidiosis infection and this may have caused the high antibody response observed in the study.
In general, our founds regarding HR to EIM might indicate the importance of intestinal homeostasis maintenance in order to express HR. Furthermore, it might indicate the relevance of adaptive immune response to the expression of HR to EIM. Also, as the genes significantly associated with HR to EIM were previously associated in literature with different nematode infections, it is possible to speculate that mechanisms developed by animals when exposed to GIN and EIM might be partially similar.

5 Conclusion

Complementary studies might be necessary to extend the conclusions we made here to other populations of different breeds or raised under different environmental conditions. Also, to replicate this study in an artificially infested population (increased environmental challenge) might help to quantify the genetic variances for HR.

The HR to GIN, and EIM can be used as a complementary tool to parasitic control management, with no negative effect over BW. Selective breeding for BW might lead to the selection of animals that are less resilient to TICK. We identified genotype x parasite burden interaction for BW so keeping the cattle exposed to controlled parasite burden, closer to the pasture parasite burden verified at commercial farms in Brazil, allows the selection of candidates that will provide heavier offspring at commercial herds.

Considering resilience as a same trait disregarding the animal age is not recommended. Further studies considering HR as a longitudinal trait are important for better definition about how the immune system’s maturity affects the mechanisms associated with HR in growing animals.

In general, and disregarding the age, the genomic regions associated with HR are mostly related to the maintenance of homeostasis when facing an infection. We recommend further studies focused on the expression of genes found here in different target tissues for a better comprehension of HR mechanisms to different parasites.

Some genes are suggested as better candidates for studying host-parasite interactions since they are apparently related to non-systemic immune mechanisms and developed to respond to each parasite individually: as YWHAE and SCARF1 for HR to TICK, AG02 for HR to GIN and TNFSF13B for HR to EIM. In general, genetic pathways evolved on the production of chemokines by intestinal epithelial cells are important for HR to gastrointestinal parasites. Further studies with other intestinal parasites can help to elucidate the important of chemokines on intestine homeostasis.
### Tables

Table 1. Summary statistics for the age of weighting, body weight (BW), ticks (TICK), gastrointestinal nematodes (GIN), and *Eimeria* spp. (EIM) counts and median of cohort parasite counts (TICK-Med, GIN-Med, and EIM-Med) at five measurement events (ME) in Nellore bulls

| Trait          | n  | mean       | sd   | Median | Min  | Max  |
|----------------|----|------------|------|--------|------|------|
| **ME.331**     |    |            |      |        |      |      |
| Age (days)     | 1539| 330.72     | 23.49| 334.00 | 275.00| 373.00|
| BW (Kg)        | 1539| 223.08     | 33.23| 220.00 | 138.00| 343.00|
| TICK           | 1539| 5.32       | 6.65 | 2.00   | 0.00 | 80.00|
| GIN            | 1539| 4.71       | 6.82 | 3.00   | 0.00 | 80.00|
| EIM            | 1539| 3.99       | 9.55 | 0.00   | 0.00 | 153.00|
| TICK-Med       | 48  | -          | -    | 3.50   | 0.00 | 16.00|
| GIN-Med        | 48  | -          | -    | 3.25   | 0.00 | 9.00 |
| EIM-Med        | 48  | -          | -    | 0.00   | 0.00 | 11.00|
| **ME.385**     |    |            |      |        |      |      |
| Age (days)     | 1214| 385.66     | 23.60| 388.00 | 339.00| 428.00|
| BW (Kg)        | 1214| 238.87     | 35.44| 237.00 | 135.00| 411.00|
| TICK           | 1214| 9.09       | 11.34| 5.00   | 0.00 | 131.00|
| GIN            | 1214| 4.93       | 6.33 | 3.00   | 0.00 | 43.00|
| EIM            | 1214| 4.50       | 14.18| 0.00   | 0.00 | 255.00|
| TICK-Med       | 40  | -          | -    | 7.00   | 0.00 | 33.00|
| GIN-Med        | 40  | -          | -    | 4.00   | 0.00 | 11.00|
| EIM-Med        | 40  | -          | -    | 2.00   | 0.00 | 10.50|
| **ME.443**     |    |            |      |        |      |      |
| Age (days)     | 1546| 443.18     | 23.69| 446.00 | 390.00| 485.00|
| BW (Kg)        | 1546| 261.21     | 36.29| 260.00 | 156.00| 380.00|
| TICK           | 1546| 5.34       | 7.36 | 3.00   | 0.00 | 80.00|
| GIN            | 1546| 5.79       | 7.64 | 3.00   | 0.00 | 80.00|
| EIM            | 1546| 3.45       | 13.27| 0.00   | 0.00 | 284.00|
| TICK-Med       | 40  | -          | -    | 2.75   | 0.00 | 15.00|
| GIN-Med        | 40  | -          | -    | 3.00   | 0.00 | 12.00|
| EIM-Med        | 40  | -          | -    | 0.00   | 0.00 | 16.50|
| **ME.498**     |    |            |      |        |      |      |
| Age (days)     | 1458| 498.23     | 23.76| 501.00 | 446.00| 541.00|
| BW (Kg)        | 1458| 305.67     | 36.84| 306.00 | 176.00| 429.00|
| TICK           | 1458| 6.24       | 8.27 | 3.00   | 0.00 | 80.00|
| GIN            | 1458| 5.13       | 6.28 | 3.00   | 0.00 | 71.00|
| EIM            | 1458| 3.63       | 12.79| 0.00   | 0.00 | 182.00|
| TICK-Med       | 44  | -          | -    | 4.00   | 0.00 | 16.00|
| GIN-Med        | 44  | -          | -    | 3.00   | 1.00 | 8.00 |
| EIM-Med        | 44  | -          | -    | 0.00   | 0.00 | 11.00|
| **ME.555**     |    |            |      |        |      |      |
| Age (days)     | 1550| 555.22     | 23.52| 558.00 | 501.00| 597.00|
| BW (Kg)        | 1550| 337.27     | 37.91| 336.00 | 214.00| 467.00|
| TICK           | 1550| 6.48       | 8.71 | 3.00   | 0.00 | 72.00|
| GIN            | 1550| 4.22       | 6.20 | 2.00   | 0.00 | 73.00|
| EIM            | 1550| 3.30       | 13.91| 0.00   | 0.00 | 328.00|
| TICK-Med       | 48  | -          | -    | 4.25   | 0.00 | 18.00|
| GIN-Med        | 48  | -          | -    | 2.75   | 0.00 | 8.00 |
| EIM-Med        | 48  | -          | -    | 0.00   | 0.00 | 7.00 |

1 n = number of observations; sd = standard deviation; min = minimum value; max = maximum value. ²The number of observations of TICK – Med, GIN – Med, and EIM – Med, are the number of evaluated cohorts in each weighting.
Table 2. Genetic parameters\(^1\) for intercept (int) and slope coefficients of body weight at five measurement events (ME)\(^2\) when ticks (TICK); gastrointestinal nematodes (GIN), and *Eimeria* spp. (EIM) burden\(^3\) was used as independent variables in single trait linear random regression models

| ME\(^3\) | \(\sigma^2_{\text{int}}\) | \(\sigma^2_{\text{slope}}\) | \(\sigma_{\text{int x slope}}\) | \(r_{\text{int x slope}}\) | \(\sigma^2_e\) |
|--------|----------------|----------------|----------------|----------------|----------------|
| 331    | 186.15         | 1.31           | -13.82         | -0.9           | 360.51         |
|        | (108; 262.4)   | (0.29; 2.19)   | (-21.25; -6.00)| (-1.00; -0.78) | (320.7; 401.6)|
| 385    | 81.05          | 0.32           | -2.14          | -0.29          | 465.09         |
|        | (9.92; 144.5)  | (0.03; 0.59)   | (-6.05; 1.99)  | (-0.92; 0.52)  | (413.7; 518.3)|
| 443    | 112.68         | 0.95           | -6.18          | -0.54          | 467.66         |
|        | (27.18; 194.8) | (0.02; 1.84)   | (-13.76; 2.38) | (-1.00; -0.01) | (416; 517.8)  |
| 498    | 145.38         | 1.09           | -6.03          | -0.45          | 527.59         |
|        | (54.55; 231.1) | (0.11; 2.03)   | (-14.03; 2.15) | (-0.97; 0.03)  | (465; 588.4)  |
| 555    | 126.41         | 1.03           | -1.68          | 0.02           | 535.91         |
|        | (43.78; 214.2) | (0.06; 1.95)   | (-9.59; 5.84)  | (0.65; 0.87)   | (470.8; 599.8)|

**TICK**

| ME\(^3\) | \(\sigma^2_{\text{int}}\) | \(\sigma^2_{\text{slope}}\) | \(\sigma_{\text{int x slope}}\) | \(r_{\text{int x slope}}\) | \(\sigma^2_e\) |
|--------|----------------|----------------|----------------|----------------|----------------|
| 331    | 163.83         | 4.53           | -19.18         | -0.66          | 341.2          |
|        | (70.57; 256)   | (1.59; 7.67)   | (-35.16; -4.15)| (-0.91; -0.45) | (297.8; 390.6)|
| 385    | 188.58         | 4.05           | -20.93         | -0.74          | 439.23         |
|        | (64.33; 301.4) | (0.97; 6.87)   | (-36.9; -4.82) | (-0.94; -0.54) | (380.9; 496.9)|
| 443    | 119.87         | 1.68           | -8.56          | -0.53          | 468.77         |
|        | (17.18; 213.5) | (0.06; 3.1)    | (-18.83; 3.83) | (-1.00; 0.08)  | (419.8; 519.4)|
| 498    | 222.44         | 6.86           | -28.18         | -0.6           | 529.12         |
|        | (8.12; 405.6)  | (0.39; 12.92)  | (-60.77; 6.38) | (-0.98; -0.17) | (468.7; 590.2)|
| 555    | 69.36          | 4.68           | 0.13           | 0.18           | 549.7          |
|        | (1.52; 130.1)  | (0.56; 8.57)   | (-13.36; 12.4) | (-0.50; 1.00)  | (485.8; 610.4)|

**GIN**

| ME\(^3\) | \(\sigma^2_{\text{int}}\) | \(\sigma^2_{\text{slope}}\) | \(\sigma_{\text{int x slope}}\) | \(r_{\text{int x slope}}\) | \(\sigma^2_e\) |
|--------|----------------|----------------|----------------|----------------|----------------|
| 331    | 105.77         | 2.65           | -6.94          | -0.33          | 341.27         |
|        | (38.78; 165.4) | (0.49; 4.51)   | (-16.35; 4.25) | (-0.83; 0.14)  | (291.7; 385.2)|
| 385    | 161.31         | 8.16           | -28.02         | -0.75          | 431.66         |
|        | (38.17; 256.3) | (2.82; 13.5)   | (-49.42; -6.6) | (-0.96; -0.53) | (375; 489.6)  |
| 443    | 74.85          | 2.23           | -2.33          | -0.07          | 462.04         |
|        | (18.39; 131.3) | (0.42; 3.92)   | (-11.18; 7.25) | (-0.80; 0.72)  | (412; 513.1)  |
| 498    | 101.27         | 3.51           | -3.21          | -0.07          | 530.03         |
|        | (23.98; 172.6) | (0.62; 6.42)   | (-15.46; 8.89) | (-0.70; 0.57)  | (470.8; 591.1)|
| 555    | 192.4          | 7.51           | -21.3          | -0.52          | 536.81         |
|        | (94.15; 292.5) | (0.96; 12.97)  | (-44.65; 1.05) | (-0.92; -0.14) | (469.8; 605.7)|

\(^1\) \(\sigma^2_{\text{int}}\) = additive genetic variance for the intercept; \(\sigma^2_{\text{slope}}\) = additive genetic variance for the slope; 
\(^2\) \(\sigma_{\text{int x slope}}\) = additive genetic covariance between intercept and slope; \(r_{\text{int x slope}}\) = genetic correlation between intercept and slope; \(\sigma^2_e\) = residual variance. \(^3\)ED are the body weight’s evaluation periods when the animals' average age were 331, 385, 443, 498, and 555 days old. \(^3\)Parasitic burden was model using information about the median infestation per cohort (contemporary group).
### Table 3. SNP derived heritability estimates for body weight (BW) and host resilience to ticks (HR.TICK), gastrointestinal nematodes (HR.GIN) and *Eimeria* spp. (HR.EIM) in different measurement events (ME)

| Trait       | ME.331        | ME.385        | ME.443        | ME.498        | ME.555        |
|-------------|---------------|---------------|---------------|---------------|---------------|
| BW          | 0.16 (0.06)   | 0.09 (0.05)   | 0.16 (0.05)   | 0.19 (0.06)   | 0.23 (0.06)   |
| HR.TICK     | 0.81 (0.04)   | 0.87 (0.04)   | 0.81 (0.04)   | 0.87 (0.03)   | 0.76 (0.04)   |
| HR.GIN      | 0.84 (0.04)   | 0.93 (0.03)   | 0.80 (0.04)   | 0.84 (0.04)   | 0.85 (0.04)   |
| HR.EIM      | 0.79 (0.04)   | 0.82 (0.04)   | 0.77 (0.04)   | 0.80 (0.04)   | 0.84 (0.03)   |

¹ME.331, ME.385, ME.443, ME.498, ME.555 are the measurement events when animals' ages were 331, 385, 443, 498 and 555 days in average.

### Table 4. Description of SNPs significantly associated with host resilience to ticks. Genes marked with bold were prioritized in the candidate gene prioritization analyses, and genes in red were not included at the prioritization analyses

| SNP                  | CHR | Position   | Genes around marker                                                                 |
|----------------------|-----|------------|--------------------------------------------------------------------------------------|
| ARS-BFGL-NGS-30621  | 2   | 130928916  | **ALPL, CDC42, ECE1, HSPG2, RAP1GAP, WNT4, CELA3B, USP48**, ENSBTAG00000030269, ENSBTAG00000040602, RF00026 |
| BovineHD1600001828  | 16  | 6430292    | **CFH, KCNT2, ENSBTAG00000040409, ENSBTAG00000048780, ENSBTAG00000049658**, ENSBTAG00000051723 |
| BovineHD1900006767  | 19  | 22882902   | **CRK, INPP5K, MNT, MYO1C, RILP, RPA1, RTN4RL1, SCARF1, SERPINF2, SERPINF1, SRR, YWHAE, WDR81, DOC2B, DPH1, HIC1, METTL16, MIR22, OVCA2, PTPNA, PRPF8, RPH3AL, SGSM2, SLC43A2, SMG6, SMYD4, TLC2D, TSR1, bta-mir-212, bta-mir-2337, bta-mir-12041, ENSBTAG00000049630, ENSBTAG00000048952, RF00026, RF00580** |

¹ SNP = peak SNPs used to define QTL, CHR = chromosome, IP = initial position; FP = final position.
### Table 5. Description of SNPs significantly associated with host resilience to gastrointestinal nematodes.

Genes marked with bold were prioritized in the candidate gene prioritization analyses, and genes in red were not included at the prioritization analyses.

| SNP                  | CHR | Position  | Genes around marker |
|----------------------|-----|-----------|---------------------|
| BTB-00384802         | 9   | 33612913  | GOPC, ROS1, KPNA5, DCBLD1, FAM162B, NUS1, RFX6, RSPH4A, SULT1C4, VGLL2, ZUP1, ENSBTAG00000050815, NEPN |
| BovineHD1400000995   | 14  | 3415584   | AGO2, KCNK9, PTK2, CHRAC1, MIR151A, TRAPPC9, RF00001, bta-mir-12027 |
| BovineHD1400002845   | 14  | 9039396   | KCNQ3, LRRC6, EFR3A, HHLA1, PHF20L1, TMEM71, ENSBTAG00000007736, ENSBTAG00000052596 |
| ARS-BFGL-NGS-119491  | 28  | 45527255  | AGT, CXCL12, COG2, TFAM, ZNF32, ZNF239, ENSBTAG00000050063, ENSBTAG00000052221 |

| SNP                  | CHR | Position  | Genes inside QTL |
|----------------------|-----|-----------|------------------|
| 4                    | 11  | 116439784 | DPP6, HTR5A, PAXIP1, RF00006 |
| 6                    | 14  | 90646323  | CXCL9, CXCL10, CXCL11, NAAA, SCARB2, STBD1, ART3, CCDC158, NUP54, PPEF2, SDA1, SHROOM3, SOWAHB, ENSBTAG00000004921, ENSBTAG00000032074, ENSBTAG00000050665, ENSBTAG00000053885, ENSBTAG00000054432, SEPT11, RF00003, RF00026 |
| 7                    | 11  | 58461990  | DPYSL3, SPINK1, SPINK5, JAKMIP2, SCGB3A2, SPINK6, STK32A, bta-mir-2284y-7, C7H5orf46, ENSBTAG00000052309, ENSBTAG00000053960, RF00026 |
| 12                   | 17  | 83457070  | COL4A1, IRS2, LIG4, TNFSF13B, ABHD13, MYO16, RF00001 |
| 13                   | 39  | 70341842  | PTPRT, ENSBTAG0000002446, RF00026 |

1 SNP = peak SNPs used to define QTL, CHR = Chromosome, IP = initial position, FP = final position.

### Table 6. Description of quantitative trait locus (QTLs) defined from SNPs significantly associated with host resilience to *Eimeria* spp.

Genes marked with bold were prioritized in the candidate gene prioritization analyses, and genes in red were not included at the prioritization analyses.

| CHR | n | IP       | FP       | Genes inside QTL |
|-----|---|----------|----------|------------------|
| 4   | 11 | 116439784| 117037674| DPP6, HTR5A, PAXIP1, RF00006 |
| 6   | 14 | 90646323 | 91785192 | CXCL9, CXCL10, CXCL11, NAAA, SCARB2, STBD1, ART3, CCDC158, NUP54, PPEF2, SDA1, SHROOM3, SOWAHB, ENSBTAG00000004921, ENSBTAG00000032074, ENSBTAG00000050665, ENSBTAG00000053885, ENSBTAG00000054432, SEPT11, RF00003, RF00026 |
| 7   | 11 | 58461990 | 59477630 | DPYSL3, SPINK1, SPINK5, JAKMIP2, SCGB3A2, SPINK6, STK32A, bta-mir-2284y-7, C7H5orf46, ENSBTAG00000052309, ENSBTAG00000053960, RF00026 |
| 12  | 17 | 83457070 | 84943864 | COL4A1, IRS2, LIG4, TNFSF13B, ABHD13, MYO16, RF00001 |
| 13  | 39 | 70341842 | 71369326 | PTPRT, ENSBTAG0000002446, RF00026 |

1 CHR = chromosome, n = number of SNPs inside QTL, IP = initial position, FP = final position.
Table 7. Description\(^1\) of QTLs associated with host resilience to *Eimeria* spp.

| CHR | \(N_{SNP}\) | \(N_{sigSNP}\) | \(N_{suppSNP}\) | \(LD_{CHR}\) | sd_{LD} | \(LD_{1-n}\) |
|-----|--------------|----------------|----------------|-------------|---------|-----------|
| 4   | 11           | 1              | 3              | 0.17        | 0.19    | 0.70      |
| 6   | 14           | 2              | 2              | 0.15        | 0.16    | 0.51      |
| 7   | 11           | 3              | 1              | 0.15        | 0.16    | 0.85      |
| 12  | 17           | 3              | 1              | 0.17        | 0.17    | 0.71      |
| 13  | 39           | 2              | 6              | 0.15        | 0.16    | 0.54      |

\(^{1}\)CHR = chromosome; \(N_{SNP}\)=number of SNPs inside QTL; \(N_{peakSNP}\)=number of significant SNPs inside QTL (associated \(P\)-values < 2.31x10^{-6}); \(N_{suppSNP}\)=number of suggestive SNPs inside QTL (associated \(P\)-values > 2.31x10^{-6} and <10^{-4}); \(LD_{CHR}\)= average linkage disequilibrium observed between SNPs of each CHR; sd_{LD}=standard deviation of LD_{CHR}; \(LD_{1-n}\)= linkage disequilibrium between first and last SNP of QTL.

7 Figures

Figure 1. Diagram explaining data collection on performance tests of pasture raised cattle in Mundo Novo farm – Brazil, between 2010 and 2018. Body weight (BW), ticks (TICK), eggs of gastrointestinal nematodes (GIN) and oocysts of *Eimeria* spp. (EIM) counts were collected in each measurement event (ME). “Age” represents the mean age that animals had at each ME. “nb” is the number of bulls evaluated at each ME and “nc” is the number of cohorts evaluated at each ME. Red arrow indicates a 70-day interval between evaluations, while blue arrows indicate a 56-day interval.

Figure 2. Additive genetic variances \((\sigma^2_a, K^2_g)\) and heritability estimates \((h^2)\) for body weight (BW) across tick (TICK), nematodes (GIN), or *Eimeria* ssp. (EIM) burden trajectory in five measurement events (ME). ME.331, ME.385, ME.443, ME.498, ME.555 are body weight’s measurement events when animals’ age was 331, 385, 443, 498 and 555 days on average.

Figure 3. SNP correlations between body weight (BW), host resilience to ticks (HR.TICK), gastrointestinal nematodes (HR.GIN), and *Eimeria* ssp. (HR.EIM) measured at five measurement events (ME - averaged animals’ age was 331, 385, 443, 498, and 555 days old). The values above the diagonal are the Pearson correlations between SNP effects (and standard errors of SNP correlations).

Figure 4. Genetic correlations between body weight of animals submitted to different ticks’ burden (TICK), evaluated in five different evaluation periods (ME.331, ME.385, ME.443, ME.498, ME.555). 331, 385, 443, 498, and 555 represent the mean ages in days that the animals had in each evaluation. The x and y-axis of each plot correspond to the ticks’ burden observed in each period (only the minimum, maximum and the three quantiles of parasite burden are presented).

Figure 5. Genetic correlations between body weight of animals submitted to different gastrointestinal nematodes’ burden (GIN), evaluated in five different evaluation periods (ME.331, ME.385, ME.443, ME.498, ME.555). 331, 385, 443, 498, and 555 represent the mean ages in days that the animals had in each evaluation. The x and y-axis of each plot correspond to the gastrointestinal nematodes’ burden observed in each period. (only the minimum, maximum and the three quantiles of parasite burden are presented).

Figure 6. Genetic correlations between body weight of animals submitted to different *Eimeria* spp.’ burden (EIM), evaluated in five different evaluation periods (ME.331, ME.385, ME.443, ME.498, ME.555). 331, 385, 443, 498, and 555 represent the mean ages in days that the animals had in each
evaluation. The x and y-axis of each plot correspond to the *Eimeria* spp.’ burden observed in each period. (only the minimum, maximum and the three quantiles of parasite burden are presented).

**Figure 7.** Posteriori means (and high-density intervals with 90% of samples) of the genetic correlations between host resilience to ticks (TICK), gastrointestinal nematodes (GIN) and *Eimeria* spp. (EIM) at different measurement events (ME) of Nellore bulls. ME.331, ME.385, ME.443, ME.498, ME.555 are evaluation periods when animals’ age were 331, 385, 443, 498, and 555 days old in average.

**Figure 8.** Manhattan plots for the meta-analysis realized with genome-wide association studies for HR to ticks (TICK) or gastrointestinal nematodes (GIN) or *Eimeria* spp. (EIM) measured at different measurement events. The dotted line (y=5.64) indicates the threshold for statistical significance. The dashed line (y=4.00) indicates the threshold for suggestive evidence of association.

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9 Ethics approval and consent to participate

This study was realized with farm-owned animals with the farmer’s approval.

10 Consent for publication

Not applicable

11 Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

12 Competing interests
Author Daniel Resende Gonçalves was employed by the company Mundo Novo farm. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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14 Authors’ Contributions

GCG: The author has worked on planning the project of study, performed the analysis and written the paper;
VMPR: The author has helped with data collection and with this paper writing;
MRSF: The author has co-supervised the data analysis and helped writing this paper;
FSSR: The author has co-supervised the data analysis and helped writing this paper;
AR: The author contributed in the statistical analysis and discussion of results;
MMM: The author has helped with data collection and with this paper writing;
AEMA: The author has helped with data collection;
DRG: The author has helped with data collection;
MVGBS: The author participated in the research funding and helped with writing this paper;
FLBT: The author participated in the planning of the project, research founding, co-supervised the data analysis, and helped writing this paper.

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