Abstract: Among the gastrointestinal nematodes affecting sheep, *Haemonchus contortus* is the most prevalent and virulent, resulting in health problems and production losses. Therefore, selecting sheep resistant to *H. contortus* is a suitable and sustainable strategy for controlling endoparasites in flocks. Here, 287 lambs of the native Brazilian Morada Nova hair sheep breed were subjected to two consecutive artificial infections with *H. contortus* and assessed for fecal egg count (FEC), packed cell volume (PCV), and live weight (LW). Forty-four animals ranked as having extreme resistance phenotypes were genotyped using the Illumina OvineSNP50v3 chip. A case–control genome-wide association study (GWAS) detected 37 significant (*p* < 0.001) markers in 12 ovine chromosomes in regions harboring quantitative trait loci (QTL) for FEC, *Trichostrongylus* spp. adults and larvae, weight, and fat; and candidate genes for immune responses, mucins, hematological parameters, homeostasis, and growth. Four single-nucleotide polymorphisms (SNP; OAR1_rs427671974, OAR2_rs419998472, OAR5_rs424070217, and OAR17_rs401006318) genotyped by qPCR followed by high-resolution melting (HRM) were associated with FEC and LW. Therefore, molecular markers detected by GWAS for *H. contortus* resistance in Morada Nova sheep may support animal selection programs aimed at controlling gastrointestinal nematode infections in flocks. Furthermore, genotyping of candidate genes using HRM qPCR may provide a rapid and efficient tool for animal identification.

Keywords: gastrointestinal nematode control; genotyping; GWAS; molecular markers; ovine; parasite resistance

1. Introduction

Morada Nova is a hair sheep breed characterized by high heat tolerance and adaptation to tropical climatic conditions [1]. Traditionally bred in the Brazilian Northeast region in small farms under extensive production systems, this native breed is also raised in other conditions and regions of Brazil [2]. Despite their small body size, these animals present interesting traits such as prolificacy, early sexual maturity, maternal ability, lack of reproductive seasonality, and resistance to gastrointestinal nematodes [3].

The barber pole worm, *Haemonchus contortus*, is the most prevalent and virulent nematode species that affects small ruminants in tropical regions [4]. *H. contortus* is a blood-sucking parasite of the abomasum, which causes anemia, weight loss, and death [5]. Considering the negative impact of nematodes on animal health and production, and the consequent economic losses [4,6], parasite resistance is an important trait that should be explored for animal selection in sheep. Furthermore, selection for resistance is a sustainable measure for parasite control [7], as it reduces production losses, reduces the use of anthelmintics, and decreases the infectiousness of pastures and the subsequent larval challenge to animals in the flock [8,9].
Several gene polymorphisms associated with resistance to gastrointestinal nematodes have been detected in Morada Nova sheep by candidate gene genotyping [10,11]; however, no genome-wide association studies (GWAS) have been identified for parasite resistance in this breed. Parasite resistance is a quantitative complex trait, and several genes may contribute to the final phenotype [12]. Therefore, GWAS can detect associated genes and polymorphisms, which may help to elucidate the genetic mechanisms affecting the inheritance of parasite resistance.

Therefore, a GWAS of H. contortus-resistant and -susceptible Morada Nova sheep was performed using a case–control design to identify genomic regions involved in resistance to gastrointestinal nematodes. Furthermore, to our knowledge, this was the first GWAS related to H. contortus resistance in a Brazilian native parasite-resistant Morada Nova sheep breed resulting in 37 significant markers located on 12 ovine chromosomes harboring regions previously associated with resistance-related traits and functional candidate genes. In addition to providing guidance for studies in other breeds, knowledge of the molecular mechanisms involved in parasite resistance will support the use and maintenance of Morada Nova sheep as a genetic stock for gene introgression and direct use in production systems, aiming to control nematode infections in flocks.

2. Results

The genome-wide association study (GWAS) identified 37 significant ($p < 0.001$) single nucleotide polymorphism (SNP) markers located on 12 ovine chromosomes (OAR 1, 2, 3, 5, 6, 7, 8, 11, 15, 17, 18, and 20) (Figure 1, Table 1, and Supplementary Table S1).

Figure 1. Manhattan plot of genome-wide association study (GWAS) for Haemonchus contortus resistance in Morada Nova sheep genotyped using the OvineSNP50v3 chip. Threshold (dashed red line) at Bonferroni $p$-value $\log_{10} \geq 3$, corresponding to significance at 0.05.
Table 1. Single-nucleotide polymorphism (SNP) markers associated ($p < 0.001$) with *Haemonchus contortus* resistance in Morada Nova sheep via genome-wide association study (GWAS). Ovine chromosomes (OAR), significance order, EMMAX $p$-value, SNP ID, SNP position (bp), superposition to functional candidate protein-coding genes, and superposition to quantitative trait loci (QTL) in the 2 Mbp upstream and downstream interval.

| OAR Order | $p$-Value | Illumina SNP ID | SNP ID | Position (bp) | Candidate Protein-Coding Genes | QTL |
|-----------|-----------|----------------|--------|---------------|------------------------------|-----|
| 1 3       | $1.02 \times 10^{-4}$ | OAR1_187356862.1 | rs427671974 | 173891949 | DPP2, CD96, CD200, BTLA, CCDC80 | Weight and fat |
| 1 2       | $7.22 \times 10^{-5}$ | s0085.1 | rs409592801 | 139163515 | KLHL41 | - |
| 2 6       | $1.32 \times 10^{-4}$ | ilmsseq_r422452493 | rs422452493 | 139188127 | - | Weight |
| 2 8       | $2.07 \times 10^{-4}$ | OAR2_154205756.1 | rs412327523 | 145234800 | KRT15 | - |
| 2 9       | $2.07 \times 10^{-4}$ | OAR2_154294235.1 | rs417376212 | 145336200 | - | Weight |
| 3 7       | $1.35 \times 10^{-4}$ | OAR2_144774839.1 | rs424565808 | 136145355 | NFE2L2, HOXD11, HOXD13, HOXD10, FFAR2, DPP4 | Weight, Trichostrongyulus spp. adults and larvae in the abomasum, and Nematospiroides FEC |
| 3 10      | $2.11 \times 10^{-4}$ | OAR3_124041988.1 | rs403393991 | 116331730 | MYF5 | Fat and T. colubriformis FEC |
| 3 11      | $2.64 \times 10^{-4}$ | OAR5_110624576.1 | rs424070217 | 101668310 | - | Weight |
| 3 31      | $6.92 \times 10^{-4}$ | s05823.1 | rs411511506 | 63148146 | GALNT10 | - |
| 3 33      | $8.59 \times 10^{-4}$ | OAR1_116227818.1 | rs398223820 | 106805224 | FER | - |
| 6 24      | $4.63 \times 10^{-4}$ | OAR6_19652340.1 | rs427107137 | 16732227 | PITX2, CFI, LEF1 | Weight and fat |
| 7 15      | $3.26 \times 10^{-4}$ | s26745.1 | rs401054470 | 46306835 | - | H. contortus FEC |
| 7 25      | $5.52 \times 10^{-4}$ | OAR7_50865088.1 | rs413854960 | 46138713 | - | Weight |
| 8 17      | $4.05 \times 10^{-4}$ | OAR8_22842614.1 | rs419418467 | 20206310 | H53ST5 | Fat and Trichostrongyulus spp. adults and larvae in the small intestine and abomasum |
| 8 40      | $4.60 \times 10^{-4}$ | OAR8_2400338.1 | rs410048009 | 21552710 | - | Weight |
| 8 41      | $4.60 \times 10^{-4}$ | OAR8_24026504.1 | rs421189130 | 21578631 | - | Weight |
| 8 42      | $4.60 \times 10^{-4}$ | OAR8_25075555.1 | rs418914462 | 22667003 | - | Weight |
| 8 37      | $8.87 \times 10^{-4}$ | OAR8_2336316.1 X.1 | rs402370166 | 2115207 | - | Weight |
| 11 18     | $4.41 \times 10^{-4}$ | OAR11_34348656.1 | rs410744616 | 32138419 | MAPK7, SREBF1 | Weight, fat, and Trichostrongyulus spp. adults and larvae in the small intestine |
| 11 19     | $4.41 \times 10^{-4}$ | OAR11_34401498.1 | rs404901308 | 32191598 | - | Weight |
| 15 13     | $3.22 \times 10^{-4}$ | OAR15_15781330.1 X.1 | rs412682230 | 15625639 | - | - |
| 15 4      | $1.19 \times 10^{-4}$ | s46114.1 | rs41006318 | 60852961 | PL2AG1B | - |
| 15 12     | $3.06 \times 10^{-4}$ | OAR17_60253864.1 | rs399621490 | 55228520 | SHB3 | - |
| 15 29     | $6.67 \times 10^{-4}$ | OAR17_60300593.1 | rs410780866 | 55270864 | EDNRA | FEC |
| 15 32     | $8.31 \times 10^{-4}$ | OAR17_12647550.1 | rs422538638 | 11361784 | - | FEC |
| 16 14     | $3.23 \times 10^{-4}$ | OAR18_51930638.1 X.1 | rs416293834 | 48701713 | TREML2, TREM1, PGC | Weight and H. contortus FEC |
| 16 27     | $5.63 \times 10^{-4}$ | OAR18_52089434.1 | s40949.1 | 48867859 | CLEC14A | - |
| 17 19     | $3.23 \times 10^{-4}$ | OAR18_52089434.1 | s40949.1 | 48867859 | CLEC14A | - |

1 In bold, SNP ID of four markers detected by GWAS and validated by qPCR followed by high-resolution melting (HRM) genotyping. 2 Functional candidates from protein-coding genes located in the 2 Mbp upstream and downstream interval.

Using Haplovieview, linkage disequilibrium (LD) blocks were detected for markers located in OAR 2 and 8, and strong LD was observed for markers in OAR 7, 11, and 18, (Figure 2).
In bold, SNP ID of four markers detected by GWAS and validated by qPCR followed by high resolution melting (HRM) genotyping.

Using Haploview, linkage disequilibrium (LD) blocks were detected for markers located in OAR 2 and 8, and strong LD was observed for markers in OAR 7, 11, and 18.

In the 2 Mbp upstream and downstream regions of each significant marker, 683 unique genes (Supplementary Table S2), including 431 protein-coding genes, were identified. The same genomic regions harbored reported quantitative trait loci (QTL; Supplementary Table S3) for *Nematodirus*, *H. contortus*, and *Trichostrongylus colubriformis* fecal egg counts (FEC), larvae and adults of *Trichostrongylus* spp. in the abomasum and small intestine, weight, and fat traits (Table 1).

Functional annotation analyses revealed enrichment of sulfotransferase activity and RNA polymerase II transcription factor activity, sequence-specific DNA binding terms for gene ontology molecular function, visual perception, response to stimulus, and phototransduction terms for biological processes, and homeobox for domain (Supplementary Table S4). Glycosaminoglycan biosynthesis–heparan sulfate/heparin, beta-alanine metabolism, histidine metabolism, and glycolysis/gluconeogenesis were enriched KEGG (Kyoto Encyclopedia of Genes and Genomes) pathways (Supplementary Tables S4 and S5).

The biological roles of genes (with localization on chromosomes) potentially involved in nematode resistance in Morada Nova sheep (Table 1) are related to the following:

- Immune response: *BTLA* (1:175586573–175619634), *CD96* (1:174690709–174790572), *CD200* (1:175479877–175498685), *CFI* (6:15708801–15753015), *DPP4* (2:146632572–146713094), *FER* (5:105044035–105461672), *LEF1* (6:17197574–17304587), *NFE2L2* (2:131754249–131759072), and the TREML1, TREM1, and TREM2 gene cluster (20:15158837–15237353);
- Gastric mucosa and mucus: *GALNT10* (5:63016723–63244040), *GCNT3* (7:47666417–47667739), and *PGC* (20:15636993–15649730);
- Hematological parameters: *CLEC14A* (18:47277443–47278737), *DLX1* (2:13659983–136602848), *EDNRA* (17:10416386–10482226), and *SH2B3* (17:54732694–54757389);
- Homeostasis: *CCDC80* (1:175729335–175762809), *HS3ST5* (8:23301492–23469323), and *PLAG1B* (17:62282828–62288877);
- Growth and muscle development: DPPA2 (1:172525876–172536184), HOXD gene cluster (2:132820016–132915560), KLHL41 (2:138842626–138856360), MYF5 (3:116620277–116622399), and PITX2 (6:14934427–14955991);
- Lipid metabolism and fat deposition: MAPK7 (11:33629196–33632861) and SREBF1 (11:34176887–34191779).

Real-time polymerase chain reaction (qPCR) followed by high-resolution melting (HRM) analyses confirmed the genotypes attributed using the chip in all 44 animals for OAR1_rs427671974, OAR2_rs419988472, OAR5_rs424070217, and OAR17_rs401006318, demonstrating 100% agreement. In addition, these four SNPs were associated with FEC (Figure 3A), and OAR1_rs427671974 and OAR2_rs419988472 were associated with live weight (LW) (Figure 3B). No association with packed cell volume (PCV) was detected for any of the four SNP markers.

Figure 3. Box plots showing the genotypic effects of SNP markers (rs427671974, rs419988472, rs424070217, and rs401006318 in OAR 1, 2, 5, and 17, respectively) on phenotypic traits in Morada Nova sheep, with p-values obtained by ANOVA. Different letters in red represent differences in group means according to Tukey’s test. (A) Mean fecal egg count (FEC). (B) Mean live weight.

3. Discussion

The present study employed a case–control GWAS for *H. contortus* resistance using OvineSNP50v3 chip genotyping of 44 ranked phenotyped (considering FEC, PCV, and LW) animals from a population of 287 Morada Nova sheep, with data from two experimental parasite challenges. GWAS detected 37 significant molecular markers, some in strong LD, on 12 ovine chromosomes in genomic regions harboring several functional candidate genes and QTLs for related traits. GWAS was validated by HRM qPCR genotyping of four significant SNP markers that were associated with FEC and LW. Mixed gastrointestinal parasite species are present under natural or field infection [12], and uninfected animals can be confounded by highly resistant animals [13]. Then, the use of two experimental parasite challenges, comprising monospecific artificial infection with *H. contortus* and repetitive phenotype collection, may have contributed to the suitability of the employed GWAS approach.

Significant markers were located in genomic regions harboring QTLs for parasite resistance, such as *H. contortus*, *T. colubriformis*, and *Nematodirus* FEC and *Trichostrongylus* spp. adults and larvae in the abomasum and small intestine, in OAR 2, 3, 7, 8, 11, 17, and 18. In addition, superposition of production trait QTLs, such as weight and fat, was detected.
in OAR 1, 2, 3, 5, 6, 8, 11, and 18. An association with production traits was expected in the present study, as live weight was used to rank animals with extreme phenotypes. However, several studies based solely on FEC and nematode infection rates have also detected regions spanning production QTLs, suggesting a correlation between live weight and growth with resistance [14], as well as a natural selection effect to ensure developmental stability under the challenge from gastrointestinal nematode infection [13]. Some genomic regions detected in GWAS for *H. contortus* resistance in Morada Nova sheep did not harbor reported QTLs for related traits, as observed for OAR 5, 15, 17, and 20, confirming that different molecular mechanisms may regulate resistance in different breeds. Consequently, due to the genetically fragmented nature of sheep and goat populations, GWAS information derived from one breed cannot be extrapolated to others without proper validation [7].

Regarding the enriched terms detected in functional analyses, homeobox has previously been associated with adaptive immune response in cattle [15], and the glycosaminoglycan biosynthesis heparan sulfate/heparin pathway has been associated with viral invasion (reviewed by [16]). In addition, changes in the metabolism of beta-alanine and other amino acids, mainly due to the effect of microbiota on the host, were observed following *H. contortus* infection [17].

Several mechanisms can disrupt parasite establishment and confer host resistance to gastrointestinal nematodes [18,19]. Briefly, while feeding in the abomasum, *H. contortus* secretes and excretes antigens that stimulate host inflammatory, humoral, and cellular immune responses. Consequently, the recruited T-helper cells release cytokines, mainly interleukins, which activate IgE synthesis, eosinophils, mast cells, and globular leukocytes in the mucosa. These events are followed by B-cell activation and antibody production (IgA and IgG1). Additionally, mast cell and eosinophil inflammatory products, such as histamines, proteases, leukotrienes, and prostaglandins, lead to mucus production and smooth muscle contraction, which induce parasitic paralysis, elimination, or death. Furthermore, events favoring host homeostasis and coping with parasitic loads, such as protein and energy metabolism, hematological parameters, and body weight, have also been associated with resistance and/or resilience to gastrointestinal nematodes [8,20]. Based on these physiological functions, candidate genes were investigated in the genomic windows 2 Mbp upstream and downstream of each significant SNP marker detected via GWAS.

Among candidate genes related to the immune response, the expression of *BTLA*, an immunoglobulin superfamily member, and *LEF1*, an enhancer of T-cell receptor-alpha, was increased in peripheral blood mononuclear cells in Suffolk sheep following exposure to *H. contortus* larvae antigens [21]. In addition, *BTLA* expression in host T CD4(+) cells and innate leukocytes affected intestinal immunity and *Strongyloides ratti* infection [22]. Increased expression of *CFI*, which regulates the complement cascade, was detected in the abomasum of *H. contortus*-resistant sheep breeds [23–25], and *CFI* was also associated with *H. contortus* FEC [26]. Increased expression of *CD96*, an immunoglobulin superfamily member, has been detected in nematode-susceptible goats [27] and cattle [28]. *CD200* [29], a glycoprotein containing two immunoglobulin domains, and *NFE2L2* (or *Nrf2*) [30], which is involved in the response to oxidative stress, affected macrophage regulation and *Leishmania* infection. *TREML1* (or *TLT-1*), *TREM2*, and *TREM1* genes, which are involved in inflammatory, innate, and adaptive immune responses, were associated with tick resistance in cattle [31], and the role of *TRML1* in clot formation and inflammatory or immune-induced bleeding has been reported [32,33]. Furthermore, increased expression of *TREM1* was detected in sheep peripheral blood mononuclear cells during chronic infection with Fasciola hepatica [34]. *FER*, a tyrosine kinase involved in leukocyte recruitment, regulated the intestinal epithelial lipopolysaccharide barrier in response to bacteria [35]. *DPP4*, which is involved in metabolism and immune regulation, was associated with the innate immune response to virus [36] and demonstrated a role in hypoxia response in sheep [37].

Regarding the gastric mucosa and mucins, the *GALNT10* gene, which drives mucin-type O-glycan synthesis, is a paralog of *GALNTL6*, which was found by GWAS to be associated with gastrointestinal parasite resistance in sheep [12]. In addition, decreased
expression of PGC, a component of the gastric mucosa, has been detected in the abomasum of a sheep breed resistant to H. contortus [23]. In cattle, increased expression of GCNT3, which plays a role in mucin-type glycoproteins, has been detected in the small intestine in response to Cooperia oncophora [38] and in the abomasum following Ostertagia ostertagi infection [39].

Considering that H. contortus is a hematophagous parasite, genes affecting hematological parameters in hosts may have a potential role in resistance. DLX1, a homeobox transcription factor, regulated the TGF-β superfamily during blood production [40]; ED-NRA, which encodes the receptor for endothelin-1, affected vasoconstriction in yaks [41]; CLEC14A controlled angiogenesis in mice [42]; and SH2B3, a negative regulator of cytokine signaling, was found to be associated with erythrocyte traits in sheep by GWAS [43].

Regarding homeostasis, PLA2G1B, a phospholipase A2 that regulates energy metabolism and inflammation in the intestine, was considered an endogenous anthelmintic that induces Heligmosomoides polygyrus and Nippostrongylus brasiliensis death in mice [44]. HS3ST5, a cell-surface heparan sulfate, acted as a receptor facilitating Trypanosoma cruzi [45] and Toxoplasma gondii [46] invasion. In addition, the expression of CCDC80, which enables glycosaminoglycan binding activity, was affected by Trypanosoma cruzi infection [47].

Some of the identified genes in this study were associated with growth, muscle development, and fat traits. Homeobox genes HOXD1, HOXD3, HOXD10, HOXD12, and HOXD13 have been detected by GWAS for muscularity in cattle [48], and PITX2 was related to growth in sheep [49] and weight in cattle [50]. DPPA2 was involved in myogenesis [51], KLHL41 was associated with skeletal muscle differentiation [52], and MYF5, a myogenic factor, was associated with growth in sheep [53]. Furthermore, MAPK7 was involved in adipocyte differentiation [54], and SREBF1 affected fat metabolism and deposition in sheep [55].

The analytical strategy employed by GWAS to detect H. contortus resistance in Morada Nova sheep was validated by association analyses of four significant SNP markers genotyped by HRM qPCR with FEC and LW in animals. No association with PCV was detected. However, while FEC and LW were used as factors multiplied by 0.4 to rank animals in extreme phenotypes, PCV presented a lower weight (0.2) in the equation. This decision was based on the fact that PCV presented lower variability among animals compared with FEC and LW, suggesting that Morada Nova sheep are resilient, rather than fully resistant to H. contortus [56]. The BB alleles of OAR1_rs427671974 and OAR2_rs41998472 were associated with lower FEC and higher LW, and the AB allele of OAR5_rs424070217 and the BB allele of OAR17_rs401006318 were associated with lower FEC. These markers, in addition to validating the GWAS results, may be used for the selection of resistant Morada Nova sheep using HRM qPCR genotyping.

4. Materials and Methods

4.1. Parasitological Tests and Phenotypic Classification of Animals

For phenotypic evaluation, 2 g of feces was collected from the rectum, mixed with 28 mL of sodium chloride-saturated solution, and evaluated in a McMaster chamber [57]. The total number of eggs was multiplied by 50 to obtain the fecal egg count (FEC). To determine packed cell volume (PCV), blood was collected into a heparin microcapillary tube and centrifuged at 1200 rpm for 5 min to determine the percentage of erythrocytes.

The DNA samples and phenotypic data (FEC, PCV, and live weight) used to rank animals with extreme phenotypes were obtained in 2017 and 2018 [58]. Briefly, 287 Morada Nova lambs (146 males and 141 females), the progeny of 7 rams from the Embrapa Pecuária Sudeste flock, were treated with monepantel (2.5 mg/kg; Zolvix®) to remove natural infection with gastrointestinal nematodes. After two FEC at 7-day intervals, animals were experimentally infected with 4000 H. contortus third-stage larvae (L3) from an anthelmintic susceptible isolate [58]. FEC was performed weekly 21, 28, 35, and 42 days post-infection (DPI), PCV was evaluated biweekly on 14, 28, and 42 DPI, and live weight (LW) was obtained on 0, 28, and 42 DPI. At 42 DPI, lambs were dewormed with monepantel (2.5 mg/kg),
and, after 15 days, they were subjected to a second parasitic challenge with 4000 L\textsubscript{3} \textit{H. contortus} of the same isolate, followed by the sampling protocol described previously. At 42 DPI of the second parasitic challenge, mean FEC, PCV, and LW were calculated from the collected data. Subsequently, animals were ranked as extreme phenotypes (extremely resistant and extremely susceptible to \textit{H. contortus}) based on the following equation:

\[
\text{Rank} = \text{LW} \times 0.4 - \text{FEC} \times 0.4 + \text{PCV} \times 0.2.
\] (1)

4.2. Genome-Wide Association Study (GWAS)

Based on phenotype ranking, 44 lambs were selected considering homogeneous phenotype distribution (21 resistant and 23 susceptible), sex (23 females and 21 males), and number of progenies (7–12) from four rams with a large number of descendants. This resulted in 12 resistant females, 11 susceptible females, 9 resistant males, and 12 susceptible males. In addition, differences in rank mean value varied from 8 to 36 times between extreme phenotype progenies from each ram.

DNA was extracted by saline precipitation [59] from venous blood samples obtained from lambs. DNA integrity was confirmed via 1% agarose gel electrophoresis, and the DNA concentration and purity (260/280 absorbance ratio between 1.8 and 2.0) were estimated using a NanoDrop 2000 spectrophotometer. The samples were genotyped using the Illumina OvineSNP50v3 chip (Supplementary Table S6) at the Centro de Genômica Funcional at ESALQ/USP in Piracicaba, SP, Brazil.

4.3. Bioinformatics and Functional Annotation

Genotyping chip data were subjected to quality control filtering, with 47,782 single-nucleotide polymorphisms (SNP) retained for analyses by case-control GWAS [60]. PLINK was used to filter markers, and SNPs were removed if they had a minor allele frequency (MAF) < 0.01, a call rate < 90%, a GC score < 0.6, and a deviation from Hardy–Weinberg equilibrium (HWE) < 10\textsuperscript{-15}. An efficient mixed-model association expedited (EMMAX) was used to identify nominal \(p\)-values for phenotype–genotype interactions, and then PLINK (using \text{–assoc}, \text{–perm}, and \text{–adjust} functions) was used for Bonferroni testing. Linkage disequilibrium (LD) between SNP markers was calculated using Haploview [61].

Genes located within a 2 Mbp window upstream and downstream of each marker were searched in the OAR v3.1 sheep genome using BioMart (www.ensembl.org/biomart accessed on 28 April 2022) and the Ensembl Genes 106 database. The same genomic interval was investigated for superposition with related sheep quantitative trait loci (QTL) mapped on SheepQTLdb (https://www.animalgenome.org/cgi-bin/QTLDb/OA/index accessed on 13 July 2022) using the Sheep Genome Track on OAR_3.1 (https://www.animalgenome.org/cgi-bin/gbrowse/sheep/ accessed on 13 July 2022).

ENSEMBL_Gene_ID was used as a gene list in the functional annotation tool of the DAVID Bioinformatics Resources 2021 update (https://david.ncifcrf.gov/home.jsp accessed on 29 April 2022) with \textit{Ovis aries} genome as the background. DAVID was used to identify the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways [62]. Considering that an enrichment score (ES) of 1.3 is equivalent to \(p = 0.05\) in Fisher’s exact test [14], ES > 1.3 was used as the threshold to detect significantly enriched terms. Classification in GeneCards version 5.9 (https://www.genecards.org/ accessed on 9 May 2022) and a literature review were used to identify functional candidate genes.

4.4. GWAS Validation

For GWAS validation, four significant SNP markers (rs427671974, rs419988472, rs424070217, and rs401006318) located on four ovine chromosomes (OAR 1, 2, 5, and 17, respectively), were genotyped via quantitative PCR (qPCR) followed by high-resolution melting (HRM) in the 44 lambs subjected to GWAS. The HRM qPCR assay consisted of 1X SsoFast Evagreen Supermix (Bio-Rad 172–5200), 0.3 \(\mu\)M of each primer (Supplementary Table S7), and 5 ng DNA in a final volume of 10 \(\mu\)L. The thermal profile on a CFX96 thermocycler
(Bio-Rad, Hercules, CA, USA) was 95 °C for 3 min, 40 cycles at 95 °C for 10 s and 60 °C for 30 s (reading), followed by melting dissociation curve analysis, from 65 to 95 °C for 10 s with 0.2 °C/5 s increments. HRM analysis was performed using Precision Melt Analysis software (Bio-Rad 184–5015).

The effects of SNPs on FEC, PCV, and LW were analyzed as described by [10]. Briefly, mean FEC values were normalized by orderNorm (ORD) using the bestNormalize package in R. Sex, age at weaning, group, ram, birth type, and age of dam were used as fixed effects, and each SNP was individually tested by ANOVA, using the aov() function, followed by Tukey’s test adjusted for unbalanced group sizes (HSD.test()).

5. Conclusions

GWAS to determine *H. contortus* resistance profiles using 44 extreme phenotype Morada Nova sheep genotyped by Illumina OvineSNP50 chip resulted in 37 significant markers in OAR 1, 2, 3, 5, 6, 7, 8, 11, 15, 17, 18, and 20. Marker genomic regions harbored QTLs for FEC, adults and larvae of *Trichostrongylus* spp. in the abomasum and small intestine, weight, and fat traits, in addition to functional candidate genes related to the immune response, gastric mucin, hematological parameters, homeostasis, growth, and fat deposition. HRM qPCR genotyping of four molecular markers (OAR1_rs427671974, OAR2_rs419988472, OAR5_rs424070217, and OAR17_rs401006318) validated their association with FEC and live weight. Thus, the obtained results can be used for the selection of native Morada Nova sheep with the aim of controlling gastrointestinal nematode infection in flocks and increasing parasite resistance in production systems.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/pathogens11080939/s1, Table S1: EMMAX p-values for phenotype–genotype interactions, Table S2: Genes located in the 2 Mbp upstream and downstream regions of each significant SNP marker on ovine chromosomes (OAR), Table S3: Quantitative trait loci (QTL) in the 2 Mbp upstream and downstream regions of each significant SNP marker on ovine chromosomes (OAR), Table S4: Enriched terms in functional annotation analyses using DAVID, Table S5: Enriched KEGG pathways, Table S6: OvineSNP50v3 Illumina, and Table S7: Fragment sizes and primers used in HRM qPCR for genotyping of SNP markers in four ovine chromosomes (OAR).

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References

1. Bueno, M.S.; Cunha, E.A.; Santos, L.E. Morada Nova: Uma Raça Com Potencial Para Produção. 2010. Available online: http://www.iz.sp.gov.br/artigo.php?id=46 (accessed on 8 July 2022).
2. McManus, C.; Facó, O.; Shiotsuki, I.; Rolo, J.L.J.P.; Peripolli, V. Pedigree analysis of Brazilian Morada Nova hair sheep. *Small Rumin. Res.* **2019**, *170*, 37–42. [CrossRef]
3. Issakowicz, J.; Issakowicz, A.C.K.S.; Bueno, M.S.; da Costa, R.L.D.; Katiki, L.M.; Geraldo, A.T.; Abdalla, A.L.; McManus, C.; Louvandini, H. Parasitic infection, reproductive and productive performance from Santa Inês and Morada Nova ewes. Small Rumin. Res. 2016, 136, 96–103. [CrossRef]

4. Amarante, A.F.T.; Bricarello, P.A.; Rocha, R.A.; Gennari, S.M. Resistance of Santa Inês, Suffolk and Ille de France sheep to naturally acquired gastrointestinal nematode infections. Vet. Parasitol. 2004, 120, 91–106. [CrossRef]

5. Amarante, A.F.T.; Sales, R.O. Control of endoparasites of sheep: A revision. Rev. Bras. Hig. San. Anim. 2007, 1, 14–36. [CrossRef]

6. Oliveira, P.A.; Ruas, J.L.; Riet-Correa, F.; Coelho, A.C.B.; Santos, B.L.; Marcolongo-Pereira, C.; Sallis, E.S.V.; Schild, A.L. Doencas parasitárias em bovinos e ovinos no sul do Brasil: Frequência e estimativa de perdas econômicas. Pesq. Vet. Bras. 2017, 37, 797–801. [CrossRef]

7. Zvínorova, P.I.; Halimani, T.E.; Muchadeyi, F.C.; Matika, O.; Riggio, V.; Dzama, K. Breeding for resistance to gastrointestinal nematodes—the potential in low-input/output small ruminant production systems. Vet. Parasitol. 2016, 225, 19–28. [CrossRef]

8. Bishop, S.C.; Stear, M.J. Modeling of host genetics and resistance to infectious diseases: Understanding and controlling nematode infections. Vet. Parasitol. 2003, 115, 147–166. [CrossRef]

9. Estrada-Reyes, Z.M.; Rae, D.O.; Mateescu, R.G. Genome-wide scan reveals important additive and non-additive genetic effects associated with resistance to Haemonchus contortus in Florida Native sheep. Int. J. Parasitol. 2021, 51, 535–543. [CrossRef]

10. Haehling, M.B.; Cruvinel, G.G.; Toscano, J.H.B.; Giralde, L.A.; Santos, I.B.; Esteves, S.N.; Benavides, M.V.; Barioni, Júnior, W.; Niciura, S.C.M.; Chagas, A.C.S. Four single nucleotide polymorphisms (SNPs) are associated with resistance and resilience to Haemonchus contortus in Brazilian Morada Nova sheep. Vet. Parasitol. 2020, 279, 109053. [CrossRef]

11. Okino, C.H.; Niciura, S.C.M.; Toscano, J.H.B.; Esteves, S.N.; Santos, I.B.; Von Haehling, M.B.; Figueiredo, A.; Oliveira, M.C.S.; Chagas, A.C.S. Oxine β-globin gene: A new qPCR for rapid haplotype identification and association with susceptibility to Haemonchus contortus infection. Vet. Parasitol. 2021, 294, 109434. [CrossRef]

12. Kaladeh, M.A.; Gibson, J.; Lee, S.H.; Gondo, C.; Van Der Werf, J.H.J. Detection of genomic regions underlying resistance to gastrointestinal parasites in Australian sheep. Genet. Sel. Evol. 2019, 51, 37. [CrossRef] [PubMed]

13. Ahbara, A.M.; Rouati, M.; Gharbi, M.; Rekik, M.; Haile, A.; Rischkowsky, B.; Mwacharo, J.M. Genome-wide insights on gastrointestinal nematode resistance in autochthonous Tunisian sheep. Sci. Rep. 2021, 11, 9250. [CrossRef] [PubMed]

14. Álvarez, I.; Fernández, I.; Soudre, A.; Traoré, A.; Pérez-Pardal, L.; Sanou, M.; Tapsoba, S.A.R.; Menéndez-Arias, N.A.; Goyache, F. Identification of genomic regions and candidate genes of functional importance for gastrointestinal parasite resistance traits in Djallonke sheep of Burkina Faso. Arch. Anim. Breed. 2019, 62, 313–323. [CrossRef] [PubMed]

15. Goyache, F.; Pérez-Pardal, L.; Fernández, I.; Traoré, A.; Menéndez-Arias, N.A.; Álvarez, I. Ancient autozygous segments subject to positive selection suggest adaptive immune responses in West African cattle. Gene 2021, 803, 145899. [CrossRef]

16. Arisan, E.D.; Dart, A.; Grant, G.H.; Arisan, S.; Çuhadaroglu, S.; Lange, S.; Uysal-Onganer, P. The prediction of miRNAs in SARS-CoV-2 genomes: Hsa-miR databases identify 7 key miRs linked to host responses and virus pathogenicity-related KEGG pathways significant for comorbidities. Viruses 2020, 12, 614. [CrossRef] [PubMed]

17. Xiang, H.; Fang, Y.; Tan, Z.; Zhong, R. Haemonchus contortus infection alters gastrointestinal microbial community composition, protein digestion and aminoacid allocations in lambs. Front. Microbiol. 2022, 12, 797746. [CrossRef] [PubMed]

18. Rainbird, M.A.; Macmillan, D.; Meuesen, E.N. Eosinophil-mediated killing of Haemonchus contortus larvae: Effect of eosinophil activation and role of antibody, complement and interleukin-5. Parasite Immunol. 1998, 20, 93–103. [CrossRef]

19. Naeem, M.; Isqbal, Z.; Roohi, N. Oxine haemonchosis: A review. Trop. Anim. Health Prod. 2021, 53, 19. [CrossRef]

20. Bishop, S.C. A consideration of resistance and tolerance for ruminant nematode infections. Front. Genet. 2012, 3, 168. [CrossRef]

21. Jacobs, J.R.; Middleton, D.; Greiner, S.P.; Bowdridge, S.A. RNA-Sequencing of ovine PBMC after exposure to Haemonchus contortus larval antigens. Parasite Immunol. 2020, 42, e12697. [CrossRef]

22. Breloer, M.; Hartmann, W.; Blankenhau, B.; Eschbach, M.L.; Pfieffer, K.; Jacobs, T. Cutting edge: The BTLA-HVEM regulatory pathway interferes with protective immunity to intestinal helminth infection. J. Immunol. 2015, 194, 1413–1416. [CrossRef] [PubMed]

23. Guo, Z.; González, J.F.; Hernandez, J.N.; McNeilly, T.N.; Corripio-Miyr, Y.; Frew, D.; Morrison, T.; Yu, P.; Li, R.W. Possible mechanisms of host resistance to Haemonchus contortus infection in sheep breeds native to the Canary Islands. Sci. Rep. 2016, 6, 26200. [CrossRef] [PubMed]

24. Toscano, J.H.B.; Okino, C.H.; Santos, I.B.; Giralde, L.A.; Haehling, M.B.; Esteves, S.N.; Chagas, A.C.S. Innate immune responses associated with resistance against Haemonchus contortus in Morada Nova sheep. J. Immunol. Res. 2019, 2019, 3562672. [CrossRef] [PubMed]

25. Zhang, R.; Liu, F.; Hunt, P.; Li, C.; Zhang, L.; Ingham, A.; Li, R.W. Transcriptome analysis unrevealed potential mechanisms of resistance to Haemonchus contortus infection in Merino sheep populations bred for parasite resistance. Vet. Res. 2015, 50, 7. [CrossRef] [PubMed]

26. Estrada-Reyes, Z.M.; Rae, O.; Postley, C.; Medrano, M.B.J.; Gutierrez, J.D.L.; Mateescu, R.G. Association study reveals Th17, Treg, and Th2 loci related to resistance to Haemonchus contortus in Florida Native sheep. J. Anim. Sci. 2019, 97, 4428–4444. [CrossRef]

27. Bhuiyan, A.A.; Li, J.; Wu, Z.; Ni, P.; Adetula, A.A.; Wang, H.; Zhang, C.; Tang, X.; Bhuyan, A.A.; Zhao, S.; et al. Exploring the genetic resistance to gastrointestinal nematode infection in goat using RNA-sequencing. Int. J. Mol. Sci. 2017, 18, 751. [CrossRef]
28. Araújo, R.N.; Padilha, T.; Zarlenaga, D.; Sonstegard, T.; Connor, E.E.; Van Tassel, C.; Lima, W.S.; Nascimento, E.; Gasbarre, L.C. Use of a candidate gene array to delineate expression pattern of cattle selected for resistance or susceptibility to intestinal nematodes. *Vet. Parasitol.* 2009, 162, 106–115. [CrossRef]

29. Rawat, A.K.; Pal, K.; Singh, R.; Anand, A.; Gupta, S.; Kishore, D.; Singh, S.; Singh, R.K. The CD200-CD200R cross-talk helps Leishmania donovani to down regulate macrophage and CD4+ CD44+ T cells effector functions in an NFκB independent manner. *Int. J. Biol. Macromol.* 2020, 151, 394–401. [CrossRef]

30. Menezes, J.P.B.; Khouri, R.; Oliveira, C.V.S.; Petersen, A.L.O.A.; Almeida, T.F.; Mendes, F.R.L.; Rebouças, A.A.D.; Lorentz, A.L.; Luz, N.F.; Lima, J.B.; et al. Proteomic analysis reveals a predominant NFE2L2 (NRF2) signature in canonical pathway and upstream regulator analysis of Leishmania-infected macrophages. *Front. Immunol.* 2019, 10, 1362. [CrossRef]

31. Otto, P.I.; Guimarães, S.E.F.; Verardo, L.L.; Azevedo, A.L.S.; Vandenplas, J.; Soares, A.C.C.; Sevillano, C.A.; Veroneze, R.; Pires, M.F.A.; Freitas, C.; et al. Genome-wide association studies for tick resistance in Bos taurus × Bos indicus crossbred cattle: A deeper look into this intricate mechanism. *J. Dairy Sci.* 2018, 101, 11020–11032. [CrossRef]

32. Jessica, M.O.; Fiorella, R.; Ocattivo, S.; Linnette, R.; Nahomy, L.; Kanth, M.B.; Bismarck, M.; Rondina, M.T.; Valance, W.A. TLT-1-controls early thrombus formation and stability by facilitating ALB3 outside-in signaling in mice. *Int. J. Adv. Res.* 2018, 6, 1143–1149. [CrossRef] [PubMed]

33. Schmoker, A.M.; Pearson, L.M.P.; Cruz, C.; Flores, L.G.C.; Branfiedl, S.; Torres, F.D.P.; Fonseka, K.; Cantres, Y.M.; Ramirez, C.A.S.; Melendez, L.M.; et al. Defining the TLT-1-interactome from resting and activated human platelets. *J. Proteomics* 2020, 215, 103638. [CrossRef] [PubMed]

34. Niedziela, D.A.; Naranjo-Lucena, A.; Molina-Hernández, V.; Browne, J.A.; Martínez-Moreno, A.; Pérez, J.; MacHugh, D.E.; Mulcahy, G. Timing of transcriptomic peripheral blood mononuclear cell responses of sheep to Fasciola hepatica infection differs from those of cattle, reflecting different disease phenotypes. *Front. Immunol.* 2021, 12, 729217. [CrossRef] [PubMed]

35. Qi, W.; Ebbert, K.V.J.; Craig, A.W.B.; Greer, P.A.; McCafferty, D.M. Absence of Fer protein tyrosine kinase exacerbates endotoxin induced intestinal epithelial barrier dysfunction in vivo. *Gut* 2005, 54, 1091–1097. [CrossRef] [PubMed]

36. Vergara-Alert, J.; van den Brand, J.M.; Widagdo, W.; Muñoz, M.; Raj, S.; Schipper, D.; Solanes, D.; Cordón, I.; Bensaid, A.; Haagmans, B.L.; et al. Livestock susceptibility to infection with middle east respiratory syndrome coronavirus. *Emerg. Infect. Dis.* 2017, 23, 232–240. [CrossRef] [PubMed]

37. Wei, C.; Wang, H.; Liu, G.; Zhao, F.; Kijas, J.W.; Ma, Y.; Lu, J.; Zhang, L.; Cao, J.; Wu, M.; et al. Genome-wide analysis reveals adaptation to high altitudes in Tibetan sheep. *Sci. Rep.* 2016, 6, 26770. [CrossRef] [PubMed]

38. Li, R.W.; Gasbarre, L.C. A temporal shift in regulatory networks and pathways in the bovine small intestine during Cooperia oncophora infection. *Int. J. Parasitol.* 2009, 39, 813–824. [CrossRef] [PubMed]

39. Rinaldi, M.; Dreesen, L.; Hoorens, P.R.; Li, R.W.; Claerebout, E.; Goddeeris, B.; Vercauteren, J.; Broek, W.V.D.; Geldhof, P. Infection with the gastrointestinal nematode Ostertagia ostertagi in cattle affects mucus biosynthesis in the abomasum. *Vet. Parasitol.* 2018, 276–282. [CrossRef] [PubMed]

40. Rawat, A.K.; Pal, K.; Singh, R.; Anand, A.; Gupta, S.; Kishore, D.; Singh, S.; Singh, R.K. The CD200-CD200R cross-talk helps Leishmania donovani to down regulate macrophage and CD4+ CD44+ T cells effector functions in an NFκB independent manner. *Int. J. Biol. Macromol.* 2020, 151, 394–401. [CrossRef]

41. Menezes, J.P.B.; Khouri, R.; Oliveira, C.V.S.; Petersen, A.L.O.A.; Almeida, T.F.; Mendes, F.R.L.; Rebouças, A.A.D.; Lorentz, A.L.; Luz, N.F.; Lima, J.B.; et al. Proteomic analysis reveals a predominant NFE2L2 (NRF2) signature in canonical pathway and upstream regulator analysis of Leishmania-infected macrophages. *Front. Immunol.* 2019, 10, 1362. [CrossRef]

42. Otto, P.I.; Guimarães, S.E.F.; Verardo, L.L.; Azevedo, A.L.S.; Vandenplas, J.; Soares, A.C.C.; Sevillano, C.A.; Veroneze, R.; Pires, M.F.A.; Freitas, C.; et al. Genome-wide association studies for tick resistance in Bos taurus × Bos indicus crossbred cattle: A deeper look into this intricate mechanism. *J. Dairy Sci.* 2018, 101, 11020–11032. [CrossRef]

30. Menezes, J.P.B.; Khouri, R.; Oliveira, C.V.S.; Petersen, A.L.O.A.; Almeida, T.F.; Mendes, F.R.L.; Rebouças, A.A.D.; Lorentz, A.L.; Luz, N.F.; Lima, J.B.; et al. Proteomic analysis reveals a predominant NFE2L2 (NRF2) signature in canonical pathway and upstream regulator analysis of Leishmania-infected macrophages. *Front. Immunol.* 2019, 10, 1362. [CrossRef]

43. Zhu, S.; Guo, T.; Zhao, H.; Qiao, G.; Han, M.; Yuan, C.; Wang, T.; Li, F.; Yue, Y.; et al. Genome-wide association study using individual single-nucleotide polymorphisms and haplotypes for erythrocyte traits in Alpine Merino sheep. *Anim. Biotechnol.* 2019, 30, 61. [CrossRef]

44. Entwistle, L.J.; Pelly, V.S.; Coomes, S.M.; Kannan, Y.; Perez-Llort, J.; Czieso, S.; Santos, M.S.; MacRae, J.I.; Collinson, L.; Sesay, M.S.; et al. Use of a candidate gene array to delineate expression pattern of cattle selected for resistance or susceptibility to intestinal nematodes. *Vet. Parasitol.* 2009, 162, 106–115. [CrossRef]

45. Calvet, C.M.; Toma, L.; Souza, F.R.; Meirelles, M.N.S.L.; Pereira, M.C.S. Heparan sulfate proteoglycans mediate the invasion of cardiomyocytes by Trypanosoma cruzi. *J. Eukaryot. Microbiol.* 2003, 50, 97–103. [CrossRef] [PubMed]

46. Bishop, J.R.; Crawford, B.E.; Esko, J.D. Cell surface heparin sulfate promotes replication of Toxoplasma gondii. *Infect. Immun.* 2005, 73, 5395–5401. [CrossRef]

47. Goldenberg, R.C.S.; Iacobas, D.A.; Iacobas, S.; Rocha, L.L.; Fortes, F.S.A.; Vairo, L.; Nagiyoithi, F.; Carvalho, A.C.C.; Tanowitz, H.B.; Spray, D.C. Transcriptomic alterations in Trypanosoma cruzi-infected cardiac myocytes. *Microbes Infect.* 2009, 11, 1140–1149. [CrossRef]

48. Doyle, J.L.; Berry, D.P.; Veerkamp, R.F.; Cathy, T.R.; Evans, R.D.; Walsh, S.W.; Purfield, D.C. Genomic regions associated with muscularity in beef cattle differ in five contrasting cattle breeds. *Genet. Sel. Evol.* 2020, 52, 2. [CrossRef]

49. Zhao, H.; He, S.; Wang, S.; Zhu, Y.; Xu, H.; Luo, R.; Lan, X.; Cai, Y.; Sun, X. Two new insertion/deletion variants of the PITX2 gene and their effects on growth traits in sheep. *Anim. Biotechnol.* 2018, 29, 276–282. [CrossRef]
50. Zhang, S.; Xu, H.; Kang, Z.; Cai, H.; Dang, R.; Lei, C.; Chen, H.; Guo, X.; Lan, X. Bovine pituitary homeobox 2 (PITX2): mRNA expression profiles of different alternatively spliced variants and association analyses with growth traits. *Gene* 2018, 669, 1–7. [CrossRef]

51. Wang, L.; Zhao, Y.; Bao, X.; Zhu, X.; Kwok, Y.K.; Sun, K.; Chen, X.; Huang, Y.; Jauch, R.; Esteban, M.A.; et al. LncRNA Dum interacts with Dnmts to regulate Dppa2 expression during myogenic differentiation and muscle regeneration. *Cell Res.* 2015, 25, 335–350. [CrossRef]

52. Pak, J.H. KLHL41 in Skeletal Muscle Development. Master’s Thesis, Boston University, Boston, MA, USA, 2019. Available online: https://open.bu.edu/handle/2144/36709 (accessed on 8 July 2022).

53. Zhang, Z.; Liu, C.; Hao, W.; Yin, W.; Ai, S.; Zhao, Y.; Duan, Z. Novel single nucleotide polymorphisms and haplotype of MYF5 gene are associated with body measurements and ultrasound traits in Grassland Short-Tailed sheep. *Genes* 2022, 13, 483. [CrossRef] [PubMed]

54. Jin, M.; Wu, Y.; Wang, J.; Chen, J.; Huang, Y.; Rao, J.; Feng, C. MicroRNA-24 promotes 3T3-L2 adipocyte differentiation by directly targeting the MAPK7 signaling. *Biochem. Biophys. Res. Commun.* 2016, 474, 76–82. [CrossRef] [PubMed]

55. Liang, C.; Qiao, L.; Han, Y.; Liu, J.; Zhang, J.; Liu, W. Regulatory roles of SREBF1 and SRBF2 in lipid metabolism and deposition in two Chinese representative fat-tailed sheep breeds. *Animals* 2020, 10, 1317. [CrossRef] [PubMed]

56. Toscano, J.H.B.; Santos, I.B.; Haehling, M.B.; Giraldelo, L.A.; Lopes, L.G.; Silva, M.H.; Figueredo, A.; Esteves, S.N.; Chagas, A.C.S. Morada Nova sheep breed: Resistant or resilient to Haemonchus contortus infection? *Vet. Parasitol. X* 2019, 2, 100019. [CrossRef]

57. Ueno, H.; Gonçalves, P.C. *Manual para Diagnóstico das Helmintoses de Ruminantes*, 4th ed.; Japan International Cooperation Agency: Tokyo, Japan, 1998; pp. 14–45.

58. Echevarria, F.M.A.; Armour, J.; Duncan, J.L. Efficacy of some anthelmintics on an ivermectin-resistant strain of Haemonchus contortus in sheep. *Vet. Parasitol.* 1991, 39, 279–284. [CrossRef]

59. Niciura, S.C.M.; Cruvinel, G.G.; Moraes, C.V.; Bressani, F.A.; Malagó Junior, W.; Benavides, M.V.; Chagas, A.C.S. PCR-based genotyping of SNP markers in sheep. *Mol. Biol. Rep.* 2018, 45, 651–656. [CrossRef]

60. Benavides, M.V.; Souza, C.J.H.; Moraes, J.C.F. How efficiently genome-wide association studies (GWAS) identify prolificity-determining genes in sheep. *Genet. Mol. Res.* 2018, 17, gmr16039909. [CrossRef]

61. Barrett, J.C.; Fry, B.; Maller, J.; Daly, M.J. Haploview: Analysis and visualization of LD and haplotype maps. *Bioinformatics* 2005, 21, 263–265. [CrossRef] [PubMed]