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Effect of *Ovar-DRA* and *Ovar-DRB1* genotype in small ruminants with *haemonchosis*

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Summary

The effect of *Ovar-DRA* and *Ovar-DRB1* genotypes on faecal egg count (FEC) was determined in sheep and goats infected with *Haemonchus contortus*. One hundred and forty-three sheep from 3 different breeds (St. Croix, Katahdin and Dorper) and 150 goats from three different breeds (Spanish, Boer and Kiko) were used. Parasitological (FEC), haematological (packed cell volume) and immunological (IgA, IgG and IgM) parameters were measured. Sheep populations showed a higher FEC and humoural response than goat breeds. Genotypes were determined by high-resolution melting assays and by conventional PCR. For *Ovar-DRA*, sheep and goats carrying the AA genotype showed significant lower FEC than AG and GG genotypes. The additive effect was found to be 115.35 less eggs per gram of faeces for the A allele for goats. For *Ovar-DRB*1, only in sheep, the GC genotype was associated with low FEC. The additive effect was 316.48 less eggs per gram of faeces for the G allele, and the dominance effect was 538.70 less eggs per gram of faeces. The results indicate that single nucleotide polymorphisms within *Ovar-DRA* and *Ovar-DRB1* could be potential markers to be used in selection programmes for improving resistance to *Haemonchus contortus* infection.

KEYWORDS
candidate gene analysis, goats, *Haemonchus contortus*, major histocompatibility complex, sheep, single nucleotide polymorphisms

1 | INTRODUCTION

Gastrointestinal nematode infection (GNI) is the most pervasive and challenging problem for the small ruminant industry worldwide. Among GNI, *Haemonchus contortus* is one of the most pathogenic parasites that has been noted.[1,2] Moreover, anthelmintic resistance in sheep and goat farms has complicated control measures of U.S. producers. In addition to the improved use of diagnostic tools such as FAMACHA or faecal egg count (FEC), genetic selection could play an important role in reducing the dependence on anthelmintic treatment by identifying resistant animals to GNI. Selection for nematode resistance in breeds of sheep and goats has been focused on indicator traits such as FEC.[3,4] Based on FEC, subtropical and tropical breeds such as St. Croix, Katahdin, Barbados Black Belly, Gulf Coast Native or Santa Ines have been characterized as moderately resistant to *H. contortus*[5–9] compared with South African or temperate breeds such as Dorper, Columbia, INRA 401 and Suffolk.[2,10,11] However, FEC phenotyping in sheep and goats for selective breeding programmes requires the exposure of the animals to gastrointestinal nematodes, and this has a negative effect on the lifetime production and subsequently will impact the economic status of producers. Thus, the use of genomic markers associated with resistance to GNI could allow for selection of animals early in life and for decreased production costs.

Evidence from candidate gene studies in several resistant breeds using the major histocompatibility complex (MHC) as a target

suggests an association of these genes to nematode resistance or low FEC.[12,13] Most of the polymorphisms within the MHC DRB1 locus of sheep (*Ovar-DRB1*) are located in the second exon,[14] which contributes directly to the diversity of bound peptides presented to CD4+ T cells.[15] In contrast with this highly polymorphic locus and in spite of the strong linkage between the two regions with no evidence of recombination, the MHC DRA locus (*Ovar-DRA)* has been characterized as less polymorphic and linked to the DRB1 locus.[16]

In this study, we tested the association between genetic polymorphisms within *Ovar-DRB1* and *Ovar-DRA* loci, and FEC in six different breeds of sheep and goat populations.

2 | MATERIALS AND METHODS

2.1 | Population

The research protocol for this study was approved by the Langston University Animal Care and Use Committee. One hundred and forty-three male sheep from three different breeds (St. Croix, Katahdin and Dorper) and 150 male goats from three different breeds (Spanish, Boer and Kiko) were used during 3 years of evaluation. All animals were approximately 4 months old. Animals within breeds were different each year and were naturally exposed to parasites since birth. Animals were progeny of dams and sires classified as resistant or moderate resistant using FEC from breeding flocks located at Langston University and six commercial farms. Animals were transferred to Langston University 2 weeks after weaning. Animal groups were housed separately in adjacent pens with automated feeders allowing free-choice access to a 15% crude protein diet. During 2 weeks of adaptation, animals were dewormed with albendazole (10 and 20 mg/kg of body weight, respectively) given 6 times and 12 hours apart and with levamisole (12 and 18 mg/kg of body weight, respectively) at the end of albendazole treatment. Anthelmintic treatment applied for adaptation period has been previously used in the animals evaluated for this study.

2.2 | Haemonchus contortus artificial infection protocol and FEC

The *H. contortus* used in our study were obtained from Dr. Tom Craig, Department of Veterinary Pathobiology, College of Veterinary Medicine, Texas A&M University. The isolate was an F2 strain from combining resistant *H. contortus* females and susceptible *H. contortus* males. Susceptible larvae were collected from a wild pronghorn antelope. Fresh faeces were collected from a donor animal with F2 monospecific infection to assay faecal cultures at 27°C for 7 days. Infective larvae were harvested using the Baermann apparatus for 8 hours.

Animals were infected with a total of 10 000 L3 larvae per oral route. Faecal samples were collected to determine FEC on day 28, 35 and 42 post-infection. The average of the measurements was used for the analysis, and units were reported as eggs per gram (EPG). Animals were dewormed at the end of the trial with the same prophylactic treatment as used at the beginning of the experiment and returned to commercial farms.

2.3 | Blood sampling

Blood samples were obtained from the jugular vein with sterile vacuum tubes with anticoagulant at day 7, 14, 21, 28 and 35 post-infection to determine packed cell volume (PCV). The average of the PCV measurements was used for the analysis. On day 21 post-infection, a duplicated blood sample per animal was collected to isolate DNA and measure immunoglobulin levels. Serum samples were stored at −20°C until immunoglobulin measurements.

2.4 | Enzyme-linked immunosorbent assays

Blood samples were centrifuged at 1500 *g* for 20 min to harvest serum for analysis of immunoglobulins (Ig). Total concentrations of IgA, IgM and IgG were determined by sandwich enzyme-linked immunosorbent assay (ELISA). All procedures for ELISA were performed using goat IgA, IgG and IgM ELISA Quantitation Set according to directions of the manufacturer (Bethyl Laboratories, Inc.; Montgomery, TX, USA). Assays were performed using 96-well plates, and 1 μL of affinity purified antibody was diluted in 100 μL coating buffer per well. Coating solution was added to the plates and incubated at 25°C for 60 minutes. Plates were washed five times with ELISA wash solution. After washing, 200 μL of blocking solution was added to each well followed by an incubation step for 30 minutes at 25°C. Then, plates were washed five times as previously described to remove the blocking solution. Dilution of the standards in sample/conjugate diluent and serum samples was performed according the manufacturer's recommendations. HRP detection antibody (Bethyl Laboratories, Inc., Montgomery, TX) was diluted in sample/conjugate diluent, and 100 μL was transferred to each well and incubated for 60 minutes at 25°C. Plates were washed, and 100 μL of substrate solution was added per well. An incubation step in the dark for 15 minutes at 25°C was carried out. Finally, reactions were stopped by adding 100 μL of ELISA stop solution. Absorbance measurements were performed on an ELISA plate reader set at 450 nm.

2.5 | DNA isolation

DNA was purified using DNeasy Blood & Tissue Kit (Qiagen, Valencia, CA) according the manufacturer's instructions. The yield was calculated from a spectrophotometric measurement at 260 nm (NanoDrop-1000, Thermo Scientific), and the purity was assessed using a ratio 260/280 nm.

2.6 | Primer design and sequencing of Ovar-DRA

Nucleotide sequences for the ovine (*Ovis aries) Ovar-DRA* gene were obtained from the Ensembl database (http://www.ensembl.org/Ovis_aries/Info/Index). For *Ovar-DRA*, oligonucleotides were designed to amplify a fragment of 172 bp containing partial intron two and exon three sequences. To identify new potential SNPs within the targeted fragment, PCRs were set up from 48 selected sheep and goats with the lowest and highest FEC. For the sample selection,

8 sheep and 8 goats from each year were selected. The reaction mixture consisted of 12.5 μL of OneTaq® 2X Master Mix with Standard Buffer (NEB, New England Biolabs), 2.5 μL of nuclease-free water, 1 μL primer mixture (10 N m) and 4 μL of DNA template. Forty cycles of amplification were performed with an initial incubation at 95°C for 7 minutes, followed by denaturation at 95°C for 30 seconds, annealing at 58°C for 30 seconds and extension at 72°C for 30 seconds. Pooled PCR samples were cleaned up using QIAquick PCR Purification Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol and sequenced. Alignments of the sequencing data were performed using CLC Sequence Viewer 7.6.1. One SNP located 10 bp upstream from the start of exon three and 2 bp upstream of a donor site (CAG) of the 3′ splice site was targeted for subsequent genotyping. An internal primer containing the SNP at the 3′ end was designed to amplify a small fragment of 41 bp flanked by the antisense primer. All primer sequences are presented in Table 1.

2.7 | Genotyping of Ovar-DRA

Real-time PCR assays were performed with 5 μL of Fast EvaGreen® qPCR Master Mix (Biotium), 1.5 μL of primer mixture (sense, internal and antisense primer) and 4 μL of DNA template per sample. Assays were performed using the Eco Real-time PCR System (Illumina). Forty cycles of amplification with an initial incubation at 57°C for 5 minutes for uracil-DNA glycosylase (UDG), followed by incubation at 95°C for 5 minutes, denaturation at 95°C for 15 seconds, annealing at 58°C for 15 seconds and extension at 72°C for 15 seconds were used. Subsequently, a high-resolution melting cycle from 55 to 95°C was performed for each plate to evaluate the melting curve of each genotype.

2.8 | Primer design and sequencing of exon 2 of Ovar-DRB1

For *Ovar-DRB1*, sheep primers described in a previous study[14] were used to amplify exon 2. To identify potential SNPs within the exon 2 of *Ovar-DRB1*, PCRs from the previously 48 selected samples were performed as described for *Ovar-DRA* but using a different annealing temperature of 62°C for 30 seconds. PCR products followed the same procedure as *Ovar-DRA* for cleaning and sequencing. From a set of potential SNPs identified from *Ovar-DRB1* sequencing data, only two SNPs were selected for further analysis because primer design was not suitable for the rest of the SNPs due to close proximity between them. Two new different forward primers were designed for amplification of the two selected SNPs. These primers differed in the 3′ end base, and an 18 bp poly-G tail was added to one of the primers. The presence of the poly-G tail allowed the discrimination of the two alleles for each SNP.

2.9 | Genotyping of Ovar-DRB1

The SNPs (SNP1 and SNP2) located in exon 2 (20:25603460 and 20:25603548, respectively) observed from the sequencing data were targeted for genotyping using conventional PCRs containing 12.5 μL of OneTaq® 2X Master Mix with Standard Buffer (NEB, New England Biolabs), 2.5 μL of nuclease-free water, 1 μL primer mixture (10 N m) and 4 μL of DNA template. Thirty-five cycles of amplification were performed with an initial incubation at 95°C for 5 minutes, followed by denaturation at 95°C for 30 seconds, annealing at 58°C for 30 seconds and extension at 72°C for 30 seconds. Polyacrylamide gels at 10% were prepared to separate PCR fragments using electrophoresis system and 1X TBE buffer. For SNP1, the allele A was 214 bp, and allele T was 232 bp. For the SNP2, the allele C was 124 bp, and allele G was 106 bp.

2.10 | Statistical analysis

Gene and genotypic frequencies were calculated using the ALLELE procedure of SAS (SAS Institute Inc., Cary, NC). The Shapiro-Wilk test was used to test all variables for normality. Box-Cox transformation was performed for the FEC phenotype, IgA, IgG and IgM levels to obtain a normal distribution of values. The R "car" library

TABLE 1 Primer sequences for the *Ovar-DRA* and *Ovar-DRB1* single nucleotide polymorphisms detection using HRM assays or conventional PCR

Gene	Primer	Sequence	Method	References
Ovar-DRA	Forward	CAACAACACCCCGAACACCAAT	qPCR HRM	
	Internal	GGACTGG**A**GCAGAGGT		
	Reverse	CGACAAGAATAGGCCCTGAGGGGAA		
Ovar-DRB1	445 FW	TATCCCGTCTCTGCAGCACATTTC	Conventional PCR	Ballingal & Tassi[14]
	229 RV	CACCCCCGCGCTCACCTCGCCGC		
Ovar-DRB1 (SNP1)	Forward	GATACTTCTATAATGGAGAAGAG**T**	Conventional PCR	
	Forward	Poly (G18) GATACTTCTATAATGGAGAAGAG**A**		
	Reverse	CACCCCCGCGCTCACCTCGCCGC		
Ovar-DRB1 (SNP2)	Forward	AGTACTGGAACAGCCAGAAGGA**C**	Conventional PCR	
	Forward	Poly (G18) AGTACTGGAACAGCCAGAAGGA**G**		
	Reverse	CACCCCCGCGCTCACCTCGCCGC		

was used to estimate the power parameter λ and carry out square root transformation for FEC (FECSQR) and logarithmic transformation (log_{10}) for IgA, IgG and IgM levels. Spearman phenotypic correlations among all the parameters evaluated were obtained through PROC CORR of SAS.

To evaluate the effect of *Ovar-DRA* and *Ovar-DRB1* genotype, a generalized linear model (GLM) was used to analyse each SNP individually to avoid collinearity and find a possible association between all the variables and genotype. The analysis was performed using PROC GLM procedure of SAS (SAS Institute Inc., Cary, NC), and the statistical significance level was defined as <.05. Least squares means were separated using the PDIFF option of GLM in SAS.

The statistical model used to evaluate the effect of *Ovar-DRA* included fixed effects of genotype (SNP), breed nested within species and year. Interactions between genotype and species and between genotype and breed were tested but were not significant. A sire model was not used because pedigree information was not available.

The general linear model used for *Ovar-DRA* was as follows:

$$Y_{ijkl} = \text{genotype}_i + \text{breed(species)}_{jk} + \text{year}_l + e_{ijkl},$$

where *Y* represents the phenotype (FECSQR, PCV, IgA, IgG or IgM), genotype is the fixed effect of the individual SNP, breed (species) is the nested effect of breed (St. Croix, Katahdin, Dorper, Spanish, Boer and Kiko) within species (sheep or goat), year is the random effect due to year (3 years of study) and e is the residual variation.

For *Ovar-DRB1*, the interaction between genotype and species was significant; therefore, the following GLM was used for each species separately:

$$Y_{ijk} = \text{genotype2}_i + \text{breed}_j + \text{year}_k + e_{ijk},$$

where *Y* represents the phenotype (FECSQR, PCV, IgA, IgG or IgM), genotype 2 is the fixed effect of SNP2, breed is the fixed effect of breed, year is the random effect due to year (3 years of study) and e is the residual variation. Effect of SNP1 and interaction between SNP1 and SNP2 were tested but were not significant.

Orthogonal 1 degree of freedom contrasts was constructed to test for additive (−1, 0 and 1) and dominance effects (1, −2 and 1).

3 | RESULTS

Least squares means and residual standard deviations for FECSQR, PCV and immunoglobulin levels are shown in Table 2.

3.1 | Faecal egg count

St. Croix sheep and Spanish goats had the lowest FEC, while Dorper and Boer goats had the highest FEC. In sheep, there was a difference in FEC between the St. Croix sheep and the other two sheep breeds ($P < .05$). For goats, Spanish had the lowest FEC and significant differences were observed between Spanish and Boer ($P = .003$). No differences were observed between Spanish and Kiko ($P = .43$).

TABLE 2 Least squares means (LSM) and standard error (SE) of faecal egg counts (FEC, square root transformed), packed cell volume (PCV), IgA (log_{10} transformed), IgG (log_{10} transformed) and IgM (log_{10} transformed) in sheep and goat populations

Trait	Specie	Breed	LSM	SE
FEC (eggs per gram)	Sheep	St. Croix	31.06[a]	2.95
		Katahdin	39.03[b]	2.92
		Dorper	44.19[b]	2.70
	Goat	Spanish	35.70[j]	2.48
		Boer	45.42[k]	2.16
		Kiko	38.58[j]	2.64
PCV (%)	Sheep	St. Croix	31.3[a]	0.40
		Katahdin	27.4[b]	0.39
		Dorper	29.5[c]	0.40
	Goat	Spanish	25.5[j]	0.42
		Boer	23.9[k]	0.36
		Kiko	27.9[l]	0.42
IgA (μg/mL)	Sheep	St. Croix	1.75[a]	0.06
		Katahdin	1.49[b]	0.06
		Dorper	1.25[c]	0.06
	Goat	Spanish	1.59[j]	0.05
		Boer	1.48[j]	0.05
		Kiko	1.51[j]	0.05
IgG (mg/mL)	Sheep	St. Croix	0.85[a]	0.03
		Katahdin	0.69[b]	0.02
		Dorper	0.75[c]	0.03
	Goat	Spanish	0.72[j]	0.03
		Boer	0.78[k]	0.02
		Kiko	0.61[l]	0.03
IgM (μg/mL)	Sheep	St. Croix	3.16[a]	0.02
		Katahdin	3.13[a]	0.02
		Dorper	3.11[b]	0.02
	Goat	Spanish	3.03[b]	0.02
		Boer	3.03[b]	0.02
		Kiko	2.87[c]	0.02

Means within variable and species grouping without a common superscript differ at $P < .05$.

3.2 | PCV

St. Croix had the highest PCV (31.3% ± 0.40), and significant differences were observed between St. Croix and Katahdin, and between St. Croix and Dorper. Goat breeds had lower values of PCV than sheep breeds. Kiko had the highest PCV across goat breeds (27.9% ± 0.42) which were significantly different from Spanish or Boer ($P < .05$).

3.3 | Immunoglobulin levels

St. Croix sheep had the highest IgA, IgG and IgM concentration. Significant differences were observed for IgA levels ($P < .05$) and IgG levels ($P = .01$) between St. Croix sheep and the other sheep breeds. IgM did not show significant differences between St. Croix and Katahdin.

Analysis of humoural immunity in goat populations revealed variable responses between breeds. There were no differences in IgA production among goat breeds. In contrast, IgG concentration among goat breeds was greatest for Boer ($P < .05$). Significant differences were observed between Kiko and the other goat breeds for IgM concentration ($P < .05$) with Spanish having the highest level.

3.4 | Spearman correlation test

Spearman correlations between the parasitological, haematological and immunological parameters are presented in Tables 3 and 4. In sheep populations, FEC was negatively correlated with PCV ($r = -0.25$) and IgA ($r = -0.35$), and positively correlated with IgG ($r = 0.10$) and IgM ($r = 0.21$). A negative correlation was observed between PCV and IgA ($r = -0.28$), and positive correlations were observed between PCV and IgG ($r = 0.32$), and IgM ($r = 0.29$). IgA had strong negative correlations with IgG ($r = -0.71$) and IgM ($r = -0.87$). Finally, IgG and IgM were positively correlated ($r = 0.77$). Many correlations in the goat populations had a different sign and magnitude compared with the sheep populations, except for FEC and PCV, FEC and IgG, and PCV and IgG. Goat populations presented negative correlations between FEC and PCV ($r = -0.29$), FEC and IgM ($r = -0.34$), PCV and IgM ($r = -0.24$), IgA and IgM ($r = -0.25$), and IgG and IgM ($r = -0.08$). Positive correlations were observed between FEC and IgG ($r = 0.20$), PCV and IgA ($r = 0.43$), PCV and IgG ($r = 0.20$), and IgA and IgG ($r = 0.23$).

3.5 | Ovar-DRA sequencing and genotype effect

From the genotyping assays, all three genotypes were observed (AA, GA, and GG). The gene and genotype frequencies at the *Ovar-DRA* locus for the two populations are presented in Table 5. The GLM analysis showed that *Ovar-DRA* genotype had a significant effect on FEC. No significant effects of the *Ovar-DRA* genotype were identified on PCV, IgA, IgG or IgM. The AA genotype was associated with lowest FEC and was significant when compared to GG genotype ($P < .05$) but no significant differences were found when compared to GA genotype. The additive effect for FEC was 115.38 EPG less for the A allele ($P < .05$), while the dominance effect was not significant.

TABLE 3 Spearman correlations between parasitological, haematological and immunological parameters evaluated in sheep breeds

	FEC	PCV	IgA	IgG	IgM
FEC	1	**−0.25**	**−0.35**	0.10	**0.21**
PCV		1	**−0.28**	**0.32**	**0.29**
IgA			1	**−0.71**	**−0.87**
IgG				1	**0.77**
IgM					1

Values in bold are statistically significant at $P < 0.05$.

TABLE 4 Spearman correlations between parasitological, haematological and immunological parameters evaluated in goat breeds

	FEC	PCV	IgA	IgG	IgM
FEC	1	**−0.29**	−0.09	0.03	**−0.34**
PCV		1	**0.43**	**0.20**	**−0.24**
IgA			1	**0.23**	**−0.25**
IgG				1	−0.08
IgM					1

Values in bold are statistically significant at $P < .05$.

3.6 | Sequencing of Ovar-DRB1 exon 2 and genotype effect

Two SNPs located in the second exon segregating in our populations were analysed by conventional PCR. The two SNPs were located 88 bp apart, and they represent missense mutations generating a purine-pyrimidine transversion (A/T and C/G, respectively). For SNP1, the conversion results in a substitution of Tyr to Asp in the amino acid sequence while SNP2 converts the amino acid sequence from Asp to Glu. In goats, no significant effect of either SNP was identified. In sheep, there was no effect of SNP1 on any variables addressed in this study, while SNP2 genotype had a significant effect ($P < .05$) on FEC and results are presented in Table 6. The CC genotype of SNP2 was associated with the highest FEC and was significantly different from GC and GG genotypes. The GG genotype was associated with the lowest FEC and was statistically different from CC ($P < .05$). The additive effect for FEC was 316.36 EPG less for the G allele ($P < .0001$), and there was a significant dominance effect of 538.28 EPG ($P < .0001$).

4 | DISCUSSION

In the present study, St. Croix sheep had the lowest FEC among sheep breeds and could be viewed as the most resistant. Similar findings were reported in the United States[17] where lower worm burden was observed by day 7 in St. Croix lambs when compared to Dorset × Finn–Rambouillet lambs.

Using this approach, St. Croix sheep breed has been previously characterized as most resistant in the United States and has shown exceptional parasite resistance when compared to Dorper, Suffolk or Katahdin sheep breeds during *H. contortus* infections or GNI.[5,18] A typical resistant sheep breed presents low FEC and exhibits a faster immune regulation driven by CD4+ T cells contributing to reduced establishment of *Haemonchus contortus*.[19,20] The resistant

TABLE 5 Gene and genotypic frequencies for *Ovar-DRA* and *Ovar-DRB1* polymorphisms

Gene, Breed	*N*	Gene frequency		Genotypic frequency		
Ovar-DRA, Sheep		A	G	AA	GA	GG
St. Croix	46	0.87	0.13	0.76	0.22	0.02
Katahdin	57	0.89	0.11	0.82	0.14	0.04
Dorper	47	0.81	0.19	0.74	0.13	0.13
Ovar-DRA, Goats		A	G	AA	GA	GG
Spanish	42	0.51	0.49	0.29	0.45	0.26
Boer	58	0.66	0.34	0.5	0.31	0.19
Kiko	43	0.78	0.22	0.7	0.16	0.14
Ovar-DRB1 SNP1, Sheep		A	T	AA	TA	TT
St. Croix	40	0.04	0.96	0.03	0.03	0.95
Katahdin	57	0.04	0.96	0.02	0.05	0.93
Dorper	46	0.05	0.95	0.04	0.02	0.93
Ovar-DRB1 SNP1, Goats		A	T	AA	TA	TT
Spanish	48	0.14	0.86	0.06	0.15	0.79
Boer	59	0.75	0.25	0.66	0.19	0.15
Kiko	45	0.47	0.53	0.31	0.31	0.38
Ovar-DRB1 SNP2, Sheep		G	C	GG	GC	CC
St. Croix	39	0.77	0.23	0.64	0.26	0.1
Katahdin	57	0.48	0.52	0.3	0.37	0.33
Dorper	46	0.53	0.47	0.48	0.11	0.41
Ovar-DRB1 SNP2, Goats		G	C	GG	GC	CC
Spanish	48	0.42	0.58	0.19	0.46	0.35
Boer	59	0.65	0.35	0.51	0.29	0.2
Kiko	45	0.61	0.39	0.51	0.2	0.29

TABLE 6 Least squares means (LSM) and standard error (SE) for square root of faecal egg count (FEC, eggs per gram) associated with genotypes at *Ovar-DRA* and *Ovar-DRB1* loci, and the additive and dominance effects

Genotype/Contrast	LSM/Estimate	SE	$P > \|t\|$
Sheep & goats, *Ovar-DRA*			
AA	35.32[a]	1.72	
GA	37.38[a]	2.86	
GG	44.30[b]	4.20	
AA vs GG	−7.30	4.90	.1375
(AA & AG) vs GG	10.74	2.99	.0004
Sheep, *Ovar-DRB1* SNP2			
CC	48.94[a]	2.80	
GC	33.37[b]	3.05	
GG	32.49[b]	1.94	
CC vs GG	23.21	5.83	.0001
(CC & CG) vs GG	−17.79	3.05	<.0001

Means within gene grouping without a common superscript differ at $P < .05$.

characteristic of St. Croix sheep can be explained by their place of origin in tropical areas where climate conditions favours the growth of gastrointestinal nematodes, before their initial importation into the United States in 1975.

Among goats, the Spanish breed had the lowest FEC. Interestingly, there was no significant difference in FEC when compared to St. Croix sheep, although higher FEC was observed in Spanish goats. Differences in FEC have been observed between sheep and goats under circumstances of common grazing in Australia and Scotland[21–23] with a higher FEC and parasite burden observed in adult goats compared with sheep. This could be explained by immediate worm expulsion which has been observed in sheep,[24] and it is not common in goats.[25]

Although experimental evidence and observations support the hypothesis of high level of parasite infection in goats, there are few goat breeds identified as resistant to GNI.[26–28]

As previously reported,[29] there is a markedly difference in the immune regulation between sheep and goat populations during GNI. In our study, PCV values for St. Croix and Dorper sheep were significantly higher than Kiko goats. Goat results for PCV could suggest a retarded immune response that varies depending on the

age and the exposure to GNI.[28] Based on this evidence, it seems that the variability observed in GNI between these two species depends on internal (genetic component) or external factors (feeding behaviour, nutrition and physiological status). Similar studies in hair sheep have reported negative moderate correlations between these two parameters, suggesting that animals with low FEC present high PCV.[30,31]

The humoural response observed in the present study was different among species and breeds. IgA production in St. Croix and Katahdin exceeded the response observed in goat breeds. Evidence has suggested a greater production of serum IgA during *H. contortus* infections in St. Croix lambs.[17] IgA plays an important role during GNI controlling the fecundity of *H. contortus* and has been negatively correlated with worm length.[13] Similar results have been observed during *Teladorsagia circumcincta* infections, showing a positive association of IgA with resistance.[32,33]

In our populations, FEC was negatively correlated with PCV and IgA. This could suggest that susceptible animals with high parasitic loads cannot develop an effective immune response while animals with low parasitic loads develop an effective immune response. Thus, it is possible that the low FEC observed in St. Croix sheep could be related to the effect of IgA on the parasite growth, development and fecundity of *H. contortus*.

Studies performed in sheep selected for resistance to GNI after challenge infection with *H. contortus*[19,34] have found similar observations, reporting high levels of serum IgG and IgA. Interestingly, IgA levels in Dorper sheep could suggest susceptibility to GNI. The IgG response was stronger in St. Croix sheep followed by those of Katahdin and Dorper sheep. For IgG, some studies suggest an association between IgG levels and resistance[10] and others relate the IgG production to nematode infection.[2]

The role of IgM antibodies during GNI has been less studied, but it is possibly the first mechanism of humoural response. In our populations, sheep IgM levels were greater than goat levels. A study conducted by Pernthaner et al[35] suggests that production of parasite-specific IgM and IgG antibodies in the intestinal lymph of Romney sheep during *Trichostrongylus colubriformis* infection is greater and faster in resistant animals than in susceptible individuals. However, in the present study, a significant moderate positive correlation between FEC and serum IgM was observed in sheep, suggesting a possible role in the primary immune responses and fixing the complement pathway. It has been demonstrated that GNI activates the alternate complement pathway, generating C3a and C5a peptides that mobilize eosinophils to the infected area and the chemo attractants secreted by *H. contortus* could reinforce the inflammatory response.[24,36] Contrary to sheep, goats had a significant negative moderate correlation between FEC and serum IgM, reinforcing the idea of differential immune mechanisms controlling GNI.

In addition to humoural response, elimination of GNI requires the recognition of excretion/secretion antigens produced by the gastrointestinal nematodes to initiate an appropriate acquired response in the host.[37] The MHC of sheep has been widely studied because its highly polymorphic feature and many association studies have focused their attention on *Ovar-DRB1* locus. Little research has been conducted regarding *Ovar-DRA* locus, which is less polymorphic. Our analysis shows for the first time an association between one polymorphism within *Ovar-DRA* loci and FEC in sheep and goat populations. One of the most interesting highlights of the present study was the identification of a polymorphism which generates a donor site (AG) for splicing of *Ovar-DRA*. The analysis of the *Ovar-DRA* sequence revealed the presence of a SNP not previously reported at the end of intron two (Figure 1). The SNP generates a conversion from adenine to guanine (A to G) and creates an AG site located near the 3′ end splice site that could possibly be recognized as an alternative donor site to CAG during splicing of mRNA.

The AA genotype has been found to be associated with lower FEC in the present study; however, further studies are required to verify if this site leads to the generation of alternative transcripts associated with low FEC.

The analysis of *Ovar-DRB1* exon 2 revealed one SNP associated with FEC and a relationship between the GC genotype and low FEC only in sheep breeds. A significant additive and dominance effect was found at this locus. Similar investigations performed in Merino and Polish sheep have described an association between exon 2 and FEC.[38,39] Studies performed with Suffolk, Romanov, Scottish Blackface, Ghezel and Pelibuey sheep have identified possible alleles within the *Ovar-DRB1* associated with low FEC[12,30,40,41] during GNI. Thus, it is possible that potential genetic markers for resistance to GNI are located in the *Ovar-DRB1* locus of sheep. Interestingly, although goats exhibited lower FEC than sheep, the SNP within

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Ovar- DRA1 CGACAAGAAT AGGCCCTGAG GGGAAAGTCT CAGTAACAAA AACCAAAGAG  50
Ovar- DRA2 CGACAAGAAT AGGCCCTGAG GGGAAAGTCT CAGTAACAAA AACCAAAGAG  50

Ovar- DRA1 CTCAAGCATC TGTTTAAAGC TACAATTCCC AAGTCTAGGA GGACTGGAGC 100
Ovar- DRA2 CTCAAGCATC TGTTTAAAGC TACAATTCCC AAGTCTAGGA GGACTGGCGC 100

Ovar- DRA1 AGAGGTACCA TTGGTGTCGG GGTGTTGTTG - AGCGCTTAA TCATGATGTC  149
Ovar- DRA2 AGAGGTACCA TTGGTGTCGG GGTGTTGTTG GAGCGCTTAA TCATGATGTC  150

Ovar- DRA1 CAGGTTGGCT  TTCATCACAG C 170
Ovar- DRA2 CAGGTTGGCT  TTCATCACAG C 171
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FIGURE 1 Alignment of the nucleotides sequences of the Ovar-DRA PCR product containing partial fragments of intron two and exon three. Horizontal rectangle denotes the AG site (donor splice site) created by the A allele. The vertical rectangle highlights the single nucleotide polymorphism (SNP) evaluated in the Ovar-DRA loci

Ovar-DRB1 associated with FEC in sheep breeds did not show a significant effect of genotype in goats, suggesting different mechanisms of development of resistance to GNI.

Overall, the differences observed between sheep and goats suggest different mechanisms of regulation of GNI and acquisition of immunity, and it is possible the immune response could have a potential role in the development of resistance in goat populations. Several studies have attempted to identify genetic markers associated with resistance and low FEC in sheep challenged with GNI in order to select resistant individuals early in life and reduce use of anthelmintic treatment in flocks. However, currently there is no functional marker available for application, but possibly potential genomic markers within the MHC of sheep could be used in future studies. The search for the discovery of genetic markers continues with special attention to goats because few studies have evaluated this species.

5 | CONCLUSION

The results observed in our study confirm previous findings, demonstrating that resistant sheep breeds such as St. Croix exhibit a greater immune response against *H. contortus* across species and sheep mounted a higher immune response than goat breeds. Potential genomic markers within *Ovar-DRA* and *Ovar-DRB1* loci associated with low FEC in sheep populations from our study could be used for sheep breeding programmes to improve selection for resistance to GNI. Goat populations have developed different mechanisms of response against GNI; thus, genetic markers available for sheep might not be appropriate. However, it is possible that the SNP found within *Ovar-DRA* locus could be applicable for goat species. Further studies are required to confirm the findings of the present study.

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AUTHOR'S CONTRIBUTIONS

Estrada-Reyes ZM and Mateescu RG performed the molecular assays and genetic analysis of the samples collected and wrote the present manuscript.

Tsukahara Y, Goetsch AL, Gipson TA, Sahlu T, Puchala R, Wang Z and Hart SP carried out the parasitological, haematological and immunological assays.

CONFLICT OF INTEREST

The authors of this manuscript have no financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the manuscript.

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