Signatures of selection for resistance to Haemonchus contortus in sheep and goats

Zaira Magdalena Estrada Reyes (zestrada@ufl.edu)
University of Florida https://orcid.org/0000-0002-1541-5749
Yoko Tsukahara
Langston University
Arthur L. Goetsch
Langston University
Terry A. Gipson
Langston University
Tilahun Sahlu
Langston University
Richard Puchala
Langston University
Raluca G. Mateescu
University of Florida

Research article

Keywords: Fst, Haemonchus contortus, signatures of selection, SNP, sheep, goats, immune response

Posted Date: April 15th, 2019

DOI: https://doi.org/10.21203/rs.2.9164/v1

Version of Record: A version of this preprint was published on October 15th, 2019. See the published version at https://doi.org/10.1186/s12864-019-6150-y.

Abstract

Background Gastrointestinal nematode infection (GNI) is the most important disease affecting the small ruminant industry in U.S. The environmental conditions in the southern United States are ideal for the survival and growth of the most pathogenic gastrointestinal nematode, Haemonchus contortus. Host genetic variation for resistance to H. contortus allows selective breeding for increased resistance of animals. This selection process increases the prevalence of particular alleles in sheep and goats and creates unique genetic patterns in the genome of these species. The aim of this study was to identify loci with divergent allelic frequencies using Fst statistic in three different breeds of sheep and goats previously exposed to H. contortus. Results Our results for sheep populations showed loci under selection on chromosomes 1, 2, 3, 6, 11, 12, 14, and 19. For goats, loci on chromosome 1, 2, 3, 5, 11, 12, 16, 18, 24 and 26 were under selection. SNPs under selection in CD86, NOS2, TGFB2 and TLR2 genes were identified in both species. Conclusions SNPs within immune response genes related to H. contortus exposure were identified under selection in sheep and goat populations. Results from this study support the hypothesis that resistance to H. contortus is likely to be controlled by many loci. Shared signatures of selection related to mechanisms of immune protection against H. contortus infection in sheep and goats could be useful targets in breeding programs aimed to produce resistant animals with low FEC.

Background

Gastrointestinal nematode infection (GNI) is one of the most prevalent health problems in sheep and goats and represents a major productivity threat for small ruminants. In particular, *Haemonchus contortus* is the most pathogenic parasite of the abomasum in humid regions around the globe. High disease incidence for both species was observed in the Southeast and Western US regions. According to the USDA National Animal Health Monitoring System (1), GNI were the primary cause of death loss in 2015, resulting in almost 87,000 goat and kid deaths in US. Similarly, 34,782 lamb and sheep deaths were associated to GNI in 2014 (2).

Recent advances in genomic research have provided tools to unravel the genetics underlying phenotypic variation in resistance to GNI (3). Host genetic variation for GNI promises great opportunities for selective breeding of sheep and goats with increased resistance to *H. contortus*. Fecal egg count (FEC) is currently the method of choice to identify resistance to GNI and is the standard phenotypic measure to achieve rapid genetic progress (4). Host resistance based on FEC is a heritable trait in both sheep and goats, with heritability estimates ranging between 0.01 to 0.65, and 0.1 to 0.33, respectively. In accordance, breeding studies of small ruminants have revealed a FEC reduction after concurrent selective breeding of naturally resistant sheep to GIN infection (5-7).

Sheep and goats were the first livestock species to be domesticated by humans and were initially used mainly for meat, rather than wool or milk (8). Natural selection and artificial selective breeding are the main driving forces shaping genetic variation across the sheep and goat genomes, and have gradually changed the phenotypes of these species. Within breeding strategies, selection increases the frequency of

particular alleles at different loci in the population and creates unique genetic patterns in the DNA sequence that can be traced back and investigated for further analyses.

Statistical methods for the analysis of signatures of selection are based on two different approaches, the detection of long haplotypes or the identification of differences in allele frequencies. The long haplotype detection method requires accurate allele assignment to one of the parental chromosomes (chromosome phasing) and ancestral allele identification, which sometimes can be a limitation when information about ancestors and pedigree is not available (9). On the other hand, genetic differentiation among groups can be computed using the *F*st method. This approach allows for identification of loci showing differences in allelic frequencies between two or more divergent populations, and therefore believed to be under selection. Highly genetic divergent loci between populations have more extreme *F*st values (greater than 0.25) than low genetic divergent loci (10), and extreme *F*st values are associated with either natural or artificial selection.

Using this approach for sheep, few loci have been identified as regions under selection for resistance or susceptibility to GNI (11), and in goats, information is even more scarce (12). Some candidate markers within *Ovar-DRA* and *Ovar-DRB* genes have been identified as possible genetic markers associated with low *H. contortus* FEC in sheep and goat populations (13). However, more knowledge is required to understand the genetic architecture underlying resistance against GNI in these species. Thus, the aim of this study was to identify immune loci (among 100 candidate genes) with divergent allelic frequencies in three different lines of sheep and goats, respectively, using the *F*st statistic.

Results

Genotyping and Quality Control

The initial SNP data set consisted of 5,346 SNPs for both sheep and goats. After quality control, the final SNP data set contained 1,339 SNPs for sheep and 1,020 SNPs for goats.

FEC descriptive statistics and population structure in sheep and goats

Dorper sheep had the highest FEC (1,475 ± 207.4 eggs per gram of feces) across breeds with Katahdin (1,087 ± 191.2) and St. Croix (969 ± 180.6) sheep, which were considered resistant in this study, presenting lower FEC. Thus, Katahdin and St. Croix breeds had 26.3% and 34.3% less eggs per gram of feces than Dorper sheep, respectively.

For goats, Boer goats had 1,548 ± 173.1 eggs per gram of feces. Kiko (936 ± 159.1) and Spanish (911 ± 150.9), categorized as resistant breeds, had 39.5% and 41.1% less eggs per gram of feces than Boer goats, respectively.

The heatmap in Figure 1A, based on the genetic relationships between individuals and among sheep breeds, shows one specific cluster per breed, with the Dorper and Katahdin breeds clustering closer. Also, the plot from principal component analysis (PCA) for sheep (1B), presented one specific cluster per breed.

The first two principal components explained 25.6 % and 18.4 % of the total variance observed in sheep, respectively.

For goats, the heatmap in Figure 2A shows one specific cluster per breed, with Boer and Kiko breeds clustering closer. The PCA plot for goats (Figure 2B) clustered the animals within breed and one specific cluster was observed per breed. PC1 and PC2 explained 21.7% and 17.2% of the total variance observed in goats, respectively.

Signatures of selection using *F*st in sheep

Information regarding ovine chromosome (OAR), gene, and position of the SNP locus under selection in sheep is presented in Table 1. *F*st values greater than 0.32 were observed in the sheep populations and within OAR1, 2, 3, 6, 11, 12 and 19 (Figure 3).

Table 1. Signatures of selection identified between resistant (Katahdin or St. Croix) and susceptible (Dorper) sheep breeds. Breeds compared (comparison), gene name, gene region, SNP name (chromosome and position), base pair substitution (SNP), mutation type (synonymous or missense), and *F*st value (extreme *F*st value) for the regions under selection.

Comparison	Gene	Region	SNP name	SNP	Mutation	*F*st
Katahdin vs Dorper (Resistant vs Susceptible)	SOCS2	Exon 2	OAR3:129558034	C/T	Synonymous (Ile → Ile)	0.623
	SOCS2	3' UTR	OAR3:129558430	G/A		0.588
	TLR10	Exon 2	OAR6:57923205	G/C	Missense (Val → Leu)	0.421
	NOS2	Exon 7	OAR11:18963484	A/G	Synonymous (Ile → Ile)	0.591
	NOS2	Exon 16	OAR11:18963494	T/C	Synonymous (Leu → Leu)	0.625
	TGFB2	3'UTR	OAR12:19965761	A/C		0.614
	TGFB2	3'UTR	OAR12:19965865	A/C		0.528
	LAMC1	Intron 19	OAR12:62193113	T/C		0.451
	LAMC1	3'UTR	OAR:62206133	A/G		0.462
	LAMC1	3'UTR	OAR:62206212	T/C		0.463
	LAMC1	3'UTR	OAR:62207058	C/T		0.499
	LAMC1	3'UTR	OAR:62207604	C/T		0.464
	LAMC1	3'UTR	OAR:62208066	G/A		0.528
	LAMC1	3'UTR	OAR:62208077	G/T		0.463
St. Croix vs Dorper (Resistant vs Susceptible)	EPS15	3'UTR	OAR1:25516496	G/A		0.362
	EPS15	3'UTR	OAR1:25517255	A/G		0.362
	EPS15	3'UTR	OAR1:25517849	G/A		0.38
	TLR4	Exon 4	OAR2:5882565	A/G	Missense (Phe → Leu)	0.37
	TLR4	Exon 4	OAR2:5883114	C/T	Missense (Asp → Asn)	0.36
	SOCS2	Exon 2	OAR3:129558034	C/T	Synonymous (Ile → Ile)	0.376
	SOCS2	3'UTR	OAR3:129558430	G/A		0.478
	STAT2	Exon 20	OAR3:162724229	A/G	Missense (His → Arg)	0.389

	CSF3	5'UTR	OAR11:39857496	G/A		0.48
	STAT5B	Exon 20	OAR11:41755713	G/A		0.393
	TGFB2	3'UTR	OAR12:62207058	A/C		0.584
	TGFB2	3'UTR	OAR12:62207604	A/C		0.447
	LAMC1	3'UTR	OAR12:62208066	G/A		0.542
	CCR3	Exon 1	OAR19:53067498	A/G	Missense (Leul → Gly)	0.356
St. Croix vs Katahdin (Resistant vs Resistant)	CD86	3'UTR	OAR1:184659051	C/T		0.381
	ITGA4	Intron 9	OAR2:127212146	G/C		0.324
	STAT2	Intron 10	OAR3:162721344	C/T		0.331
	STAT2	Exon 20	OAR3:162724229	A/G	Missense (His → Arg)	0.354
	IL2RB	Exon 10	OAR3:180152504	T/C	Missense (Ile →Val)	0.458
	C3AR1	3'UTR	OAR3:206099484	G/C		0.363
	TLR10	Exon 2	OAR6:57921483	T/C	Missense (Tyr → Ala)	0.33
	NOS2	Exon 6	OAR11:18975484	T/C	Synonymous (Leu → Leu)	0.363
		Exon 16	OAR11:18963494	A/G	Synonymous (Ile → Ile)	0.378
	STAT5B	Exon 20	OAR11:41755713	G/A		0.349

For the Katahdin vs Dorper analysis (Figure 3A), OAR3, 6, 11 and 12 contained extreme *F*st values within *SOCS2*, *TLR10*, *NOS2*, *TGFB2*, and *LAMC1* genes. The 3'UTR of *LAMC1* gene was the genomic region with most loci under selection. The highest *F*st values were observed for two SNPs in *SOCS2* gene (0.623 and 0.588), two SNPs within *NOS2* gene (0.591 and 0.625), and two SNPs in *TGFB2* gene (0.614 and 0.528). The SNPs within exon 2 of *SOCS2* gene (C/T) and exon 7 and 16 of *NOS2* gene (A/G) are synonymous mutations. The SNP (G/C) located on exon 2 of *TLR10* gene generates a missense mutation which confers an aminoacid substitution from valine to leucine.

For the St. Croix vs Dorper analysis (Figure 3B), loci within OAR1, 2, 3, 11, 12 and 19 were observed under selection. The genes showing high genetic differentiation were *EPS15*, *TLR4*, *SOCS2*, *STAT2*, *CSF3*, *STAT5B*, *TGFB2*, *LAMC1*, and *CCR3*. The highest *F*st values (0.584 and 0.542) were found in SNPs

located in the 3'UTR region of *TGFB2* and *LACM1* genes. These two SNPs were also observed under selection in the Katahdin vs Dorper analysis. Two SNPs within *TLR4* (A/G and C/T) were identified as missense mutations that generate aminoacid substitutions from phenylalanine (F) to leucine (L), and aspartic acid (D) to asparagine (N), respectively. Also, two SNPs within *STAT2* (A/G) and *CCR3* (A/G) genes generate missense mutations that confer aminoacid substitutions from histidine to arginine, and leucine to glycine, respectively.

For the St. Croix vs Katahdin analysis (Figure 3C), ten loci were identified with divergent allelic frequencies. SNPs within exonic regions in *CD86*, *ITGA4*, S*TAT2*, *IL2RB*, *C3AR1*, *TLR10*, *NOS2*, and *STAT5B* genes were found with high genetic divergence. The highest peak was observed in exon 10 of *IL2RB* gene (*F*st = 0.458). Loci within intronic regions in *ITGA4*, *STAT2*, and *NOS2* genes were observed under selection. The two SNPs observed in exon 10 of *IL2RB* gene and exon 2 of *TLR10* gene both conferred a base pair change from T to C and generate missense mutations (from isoleucine to valine, and from tyrosine to alanine, respectively). Also, a SNP in exon 20 of *STAT2* gene generates another missense mutation that confers an aminoacid substitution from histidine to arginine. Synonymous mutations were observed in loci under selection in *STAT5B* gene.

Signatures of selection using *F*st in goats

Information regarding loci under selection in goats is presented in Table 2 and Figure 4. For goats, genes within OAR1, 2, 3, 5, 11, 12, 16, 18, 24 and 26 contained SNPs under selection (Figure 4). Genes with loci under selection in goat populations were *CD86*, *TLR4*, *IL33*, *IGF1*, *CSF2*, *NOS2*, *LRP8*, *CD1D*, *FCER1A*, *IL12A*, *TGFB2*, *IL16*, *IL4R*, *TLR3*, *UBE2N*, and *IL7R*. Contrary to sheep, no intronic loci under selection were observed, and the majority of the SNPs generate missense and synonymous mutations.

Table 2. Signatures of selection identified between resistant (Kiko or Spanish) and susceptible (Boer) goat breeds. Breeds compared (comparison), chromosome (OAR), gene name, gene region, SNP name (chromosome and position), base pair substitution (SNP), mutation type (synonymous or missense), and *F*st value (extreme *F*st value) for the regions under selection.

Comparison	Gene	Region	SNP name	SNP	Mutation	*Fst*
Kiko vs Boer (Resistant vs Susceptible)	CD86	Exon 2	OAR1:184658290	C/T		0.53
	TLR4	Exon 4	OAR2:5881934	T/C	Missense (Asn → Ser)	0.4
	TLR4	Exon 4	OAR2:5882239	G/A	Sinonymous (His → His)	0.404
	TLR4	Exon 4	OAR2:5882350	C/T	Sinonymous (Leu → Leu)	0.4
	TLR4	Exon 4	OAR2:5882465	C/G	Missense (Ser → Thr)	0.4
	IL33	Exon 10	OAR2:73814469	A/G	Sinonymous (Thr → Thr)	0.451
	IGF1	3'UTR	OAR3:171038930	T/C		0.424
	CSF2	5'UTR	OAR5:19906108	C/T		0.483
	NOS2	5'UTR	OAR11:18986931	T/A		0.436
Spanish vs Boer (Resistant vs Susceptible)	LRP8	Exon 3	OAR1:27516627	G/A	Sinonymous (Asn → Asn)	0.325
	CD1D	Exon 3	OAR1:106993424	T/G	Missense (Ser → Pro)	0.619
	FCER1A	Exon 1	OAR1:108440407	A/G	Missense (Ile → Val)	0.354
	FCER1A	3'UTR	OAR1:108446049	T/C		0.327
	IL12A	5'UTR	OAR1:225345132	T/C		0.339
	CSF2	5'UTR	OAR5:19906108	C/T		0.35
	NOS2	5'UTR	OAR11:18993321	T/G		0.378
	TGFB2	3'UTR	OAR12:19964183	T/G		0.433
	IL16	Exon 16	OAR18:26110594	A/T	Missense (Thr → Ser)	0.349
	IL4R	Exon 6	OAR24:24903381	G/A	Missense (Ala → Thr)	0.341
	TLR3	5'UTR	OAR26:14710493	G/C	mRNA	0.354
Spanish vs Kiko (Resistant vs Resistant)	IL12A	5'UTR	OAR1:225345132	T/C		0.427
	TLR4	5'UTR	OAR2:5880687	A/C		0.364

	TLR4	Exon 4	OAR2:5881934	T/C	Missense (Asn → Ser)	0.448
	TLR4	Exon 4	OAR2:5882130	A/G	Sinonymous (Leu → Leu)	0.436
	TLR4	Exon 4	OAR2:5882239	G/A	Sinonymous (His → His)	0.436
	TLR4	Exon 4	OAR2:5882465	C/G	Missense (Ser → Thr)	0.448
	TLR4	Exon 4	OAR2:5882350	C/T	Sinonymous (Leu → Leu)	0.45
	IL33	Exon 10	OAR2:73814469	A/G	Sinonymous (Thr → Thr)	0.381
	UBE2N	5'UTR	OAR3:129412290	G/A		0.412
	IL7R	3'UTR	OAR16:38223278	C/T		0.352
		Exon 5	OAR16:38228212	G/A	Missense (Pro → Ser)	0.352

For the Kiko vs Boer analysis (Figure 4A), *CD86*, *TLR4*, *IL33*, *IGF1*, *CSF2*, and *NOS2* genes had loci under selection. The SNP within *CD86* gene observed in St. Croix vs Katahdin analysis was found under selection in Kiko vs Boer analysis. This SNP (C/T) had the highest *F*st value (0.529) and was located in the untranslated region. Also, exon 4 of *TLR4* gene contained four SNPs under selection. Two of these SNPs (T/C and C/G, respectively) generate missense mutations and the other two SNPs (G/A and C/T, respectively) generate synonymous mutations. SNPs observed under selection within *IGF1*, *CSF2*, and *NOS2* genes were identified in the untranslated regions.

For the Spanish vs Boer analysis (Figure 4B), *LRP8*, *CD1D*, *FCER1A*, *IL12A*, *CSF2*, *NOS2*, *TGFB2*, *IL16*, *IL4*, and *TLR3* genes contained loci under selection. The SNP (G/A) observed under selection in exon 3 of *LRP8* gene generates a synonymous mutation. SNPs within exon 1, 3, 6, and 16 of *CD1D*, *FCER1A*, *IL4R* and *IL16* genes, respectively, generate missense mutations. Loci under selection in *IL12A, CSF2, NOS2, TGFB2*, and *TLR3* genes were identified in the untranslated regions.

Finally, for the Spanish vs Kiko analysis (Figure 4C), SNPs within *IL12A*, *TLR4*, *IL33*, *UBE2N*, and *IL7R* genes were observed under selection. The highest *F*st values were observed in SNPs located on exon 4 of *TLR4* gene. In the same region, two SNPs (T/C, and C/G) within *TLR4* gene generate missense mutations (from asparagine to serine, and from serine to threonine, respectively). For IL7R gene, one SNP (G/A) within exon 5 generate a missense mutation (from proline to serine). Loci under selection in *IL12A* and *UBE2N* genes were identified in the untranslated regions.

Genomic regions under selection in both species

After examination of the *F*st results per species, several loci in *NOS2*, *TLR4*, *CSF*, and *CD86* genes were observed under selection in both species and are presented in Table 3 and Figure 5. For *TLR4* and *CD86* genes, SNPs under selection were found within the same exonic regions for sheep and goats.

Table 3. Common signatures of selection identified in sheep and goats. Chromosome (OAR), gene name, gene region and gene cellular function.

OAR	Gene	Region	Function
1	CD86	Exon 2	Antigen presentation /Cell activation
2	TLR4	Exon 4	Cell activation
5, 11	CSF2, CSF3	5'UTR	Cytokine/ Differentiation of granulocytes and macrophages
11	NOS2	5'UTR, Exon 16	Synthesis of nitric oxide/ regulator of macrophage functions

Discussion

Domestication, breed formation, and selective breeding leave detectable patterns within the genome of livestock species such as sheep and goats. Identification of these genomic patterns in the DNA sequence could help to identify of genes controlling resistance to *H. contortus* or other gastrointestinal parasites. Several studies have attempted to identify the genetic variation controlling gastrointestinal parasite resistance in sheep and goats by using SNP markers and Genome Wide Association Studies (GWAS) but few research studies has been devoted to identify signatures of selection for GNI resistance in these species (14-16). Signatures of selection for resistance to GNI have not been identified in goats, and for sheep, only Perendale and Romney breeds have been evaluated (11). In the present study, we unravel signatures of selection using a targeted sequencing approach in three different breeds of sheep and goats. The SNPs potentially under selection identified in this study spanned a myriad of candidate genes related to immune response and cellular mechanisms against *H. contortus*.

Signatures of selection in sheep populations

Results from these analyses revealed that *SOCS2*, *LAMC1*, *EPS15* and *TGFB2* genes contain SNPs under selection in Dorper sheep when compared to Katahdin and St. Croix breeds. The same genes have been previously associated with FEC in Dorper x Red Maasai sheep using GWAS and expression of these candidate genes was observed in abomasal tissue, mesenteric lymph nodes, and Peyer's patches from ewes and lambs (15). Thus, it is possible that *SOCS2*, *LAMC1*, and *EPS15* genes could be used as candidate genes for future studies to validate previous and current results in Dorper and Dorper × Red Maasai sheep.

The *SOCS2* gene is a broad key regulator of cytokine responses, including IL2, IL3, IL4, IFN-γ, CSF, and Jak-STAT signaling pathways in bone marrow and T cells (17). Studies on mice infected with

Tripanozoma cruzi have shown that expression of *SOCS2* facilitates inflammatory and immune responses to prevent myocardial dysfunction but increases parasitemia (18). On the contrary, *SOCS2*-/- mice infected with *Schistosoma mansoni* expressed increased Th2 response with higher IgE and eosinophil production than *SOCS2*+/+ mice (19). Also, SOCS2-/- mice have shown increased body weight and gigantism possibly due to downregulation of growth hormone and insulin-like growth factor-I (IGF-I) signaling (20). In Scottish Blackface sheep infected with *Teladorsagia circumcincta*, *SOCS2* gene was found differentially expressed between resistant and control animals (21).

The *LAMC1* gene is part of extracellular matrix (ECM) glycoproteins implicated in cell adhesion, differentiation, migration, and other cellular processes. The role of *LAMC1* during *H. contortus* infections has not been elucidated in detail but it has been shown that some parasites regulate and use the extracellular matrix components such as LAMC1 proteins to invade cells and cause disease. For example, previous studies with *T. cruzi* have evidenced its ability to upregulate *LAMC1* and recruit more parasites at the ECM to enhance infection (22). Thus, it is possible that similar action mechanisms are used by gastrointestinal parasites but more research needs to be done to clarify the role of *LAMC1* during *H. contortus* exposure.

The *EPS15* gene is involved in cell growth regulation and endocytosis. As *LAMC1* gene, the role of this gene during *H. contortus* exposure is unknown but evidence suggests that *EPS15* gene could play a key role in resistant animals. Results from microarray analysis in Angus yearlings infected with *Ostertagia, Cooperia* and *Nematodirus* spp., have shown that *EPS15* is more highly expressed in the mesenteric lymph nodes of resistant animals than susceptible individuals (23).

TGFB2 protein has been reported as an anti-inflammatory cytokine, and was observed in high concentration in the gut mucosa of sheep after *H. contortus* infection (24). In pigs, PAS1, a product of *Ascaris suum,* induces IL-10 and TGFB2 production in macrophages and has been related to loss of pro-inflammatory cytokine production (25). In humans and animal models, it has been shown that inhibition of T-cell proliferation might be triggered by an increase of IL-10 and TGFB production in antigen presenting cells or T-cells as a result of down-modulatory molecules that are released by the parasites to enhance survival (26). Thus, parasites are prone to use IL-10 and TGFB to downregulate host immune response.

In our Dorper sheep, SNP within *TLR10* (from the Katahdin vs Dorper analysis) and *TLR4* (from the St. Croix vs Dorper analysis) genes contained SNPs under selection. Evidence from previous findings suggests that Toll like-receptor (*TLR*) genes could play an important role in resistance against *H. contortus*. Previous studies have observed higher expression of these genes in resistant Merino sheep during innate and acquired infections with *H. contortus* and *Trichostrongylus columbriformis* (27). Excretion and secretion antigens from helminths can be rich in glycoproteins and lipids which can be easily recognized by TLRs in dendritic cells. In murine models, recognition of some helminth glycans is done by TLR4 dependent mechanisms (28). Thus, it is possible that stimulation of TLR pathways depends on host-parasite relationship.

For Katahdin sheep, loci within *NOS2* and *TLR10* genes where observed under selection (in both Katahdin vs Dorper and Katahdin vs St. Croix analyses). Nitrogen oxygen synthase 2 or *NOS2* is a key molecule involved in Th1 response. It participates in the production of nitric oxide to kill invading microbes in phagocytes during classical macrophage activation by IFN-γ and TNF-α. Differential expression of these genes has been observed in the abomasum of Merino sheep during *H. contortus* challenge (27). In that study, mRNA expression of *TLR10* gene was up-regulated in resistant sheep and mRNA expression *NOS2* gene was downregulated in susceptible individuals. While there is a proposed interplay between Th1, Th2, and Treg responses during GNI in sheep (29), susceptibility to these infections has been related to Th1 and Th17 responses, and Th2 has been associated with resistance to helminth infections (30).

For St. Croix sheep, SNPs within transcription factor genes (*STAT2* and *STAT5B*) were found to be under selection. The *STAT5* gene can be activated by many cytokines such as GM-CSF and thymic stromal lymphopoietin (TSLP) in the dendritic cells. Activation of *STAT5* by TSLP has been shown to trigger Th2 responses at barrier surfaces (31). Also, *STAT5* signaling has been related to many biological processes, such as TCR signaling and basal proliferation of naïve CD4+ T cells (32). Moreover, STAT5B mediates the signal transduction of IL2, IL4, CSF1, and different growth hormones. The mRNA expression of *STAT2* has been upregulated in Suffolk sheep relative to Texel sheep previously exposed to *T. circumcincta*, *Nematodirus battus*, and other Trichostrongyle spp (33). Thus, it is possible that *STAT5B* and *STAT2* genes are responsible for many cellular functions during *H. contortus* exposure and further analysis is required to confirm our results.

Signatures of selection in goat populations

For many years, there has been a debate about the immune mechanisms controlling *H. contortus* infections in sheep and goats. The same helminth species can parasitize both species but evidence suggests higher levels of infection in goats (34).

For Boer goats, *CSF2* and *NOS2* genes were observed under selection. Evaluation of the functional mechanisms of these genes has been done in sheep, but for goats, research is scarce. CSF2 and NOS2 proteins are key regulators of macrophage function and Th1 response, and we hypothesize similar mechanisms of action during *H. contortus* infections.

For Kiko goats, SNP loci within *TLR4* and *IL33* genes were found under selection. IL33 is an important nuclear cytokine from the IL1 family probably involved in the control of parasites. Studies indicate that this cytokine induces IL5 and IL13 by innate lymphoid cells to initiate inflammatory responses (35) and trigger Th2 cytokine production. Also, new interactions between IL33 and dendritic cells have been documented and may represent different pathways to elicit Th2 response (36).

Finally for Spanish breed, a SNP within *IL12A* gene was observed under selection between Spanish vs Boer, and Spanish vs Kiko. IL12 is a major cytokine responsible for the induction of Th1 responses during nematode infections (37, 38). It drives the differentiation of naïve CD4+T cells to Th1 cells. IL12 signaling

involves the activation of several transcription factors such as TBX21 to promote production of IFN-γ during *T. circumcincta* infections (39).

Common signatures of selection in sheep and goats

During the last two decades, results have shown differences in feeding behavior and gastrointestinal nematode parasitism between sheep and goats. Feeding behavior is one important aspect that differentiates sheep and goats. Sheep are typically raised in grazing systems with parasites commonly found within the pastures, and have to counteract the negative effects of GNI by developing an effective immune response. Goats are common browsers which allows them to rely less on immune response mechanisms (40).

For many years, there has been a question of the importance of immune effector molecules and the mucosal response in goats during GNI. Our findings suggest a possible interplay between Th1 and Th2 responses with conserved breed specific mechanisms. For example, CSF2 and NOS2 (Th1 response) were associated with Boer goats. For Kiko, TLR4 and IL133 were observed as conserved Th2 response mechanisms. For Spanish goats, IL12A, an inductor cytokine of Th1 response, was found as a conserved mechanism under selection. For sheep, our evidence suggests a possible interplay between Th1 and Th2 response during GNI, as previously described by Hassan et al. (30) and Pernthaner et al. (41).

One of the most interesting findings from this study is the identification of shared immune response mechanisms between sheep and goats (Figure 5). It is possible that some immune response mechanisms are shared between both species and induce an effective immune response against *H. contortus.* The *NOS2*, *CSF*, *TLR4*, and *CD86* genes, observed under selection in both species, are key modulators of Th1 and Th2 responses, and active players of antigen recognition by dendritic cells and T-cells. Several studies in cashmere and dairy goats have evaluated the responsiveness of resistant animals to GNI and have found a negative correlated response between worm counts and eosinophil, mast cell, and globule leucocyte counts (42 - 44). In sheep, similar cellular immune response has been associated to GNI (45, 46).

The conserved mechanisms of protective response against *H. contortus* are most likely to be useful targets in the development of alternative nematode control strategies in both species, as they can be widely applied in production systems. For this reason our future efforts will focus on validation of the results observed in the present study to unravel genetic mechanisms used for controlling *H. contortus* or other GNI between sheep and goats.

Conclusion

Results from this study support the hypothesis that resistance to gastrointestinal parasites such as *H. contortus* is likely to be controlled by many loci. Different immune response mechanisms between sheep and goats are used to control *H. contortus* but some aspects are shared in both species. Shared

mechanisms of immune protection could be useful targets in breeding programs aimed to produce resistant animals and future research is necessary to validate our findings.

Methods

Animal populations

The research protocol for the present study was approved by the Langston University Animal Care and Use Committee. One hundred and twenty sheep from Dorper (n=40), Katahdin (n= 40), and St. Croix (n=40) breeds and 132 goats from Boer (n=44), Kiko (n=44) and Spanish (n=44) breeds from commercial farms in Arkansas, Kansas, and Missouri and American Institute for Goat Research, Langston University Oklahoma, were used in this study. In the first year, weaned males under study were randomly selected from each farm and transferred to Langston University for a central sire performance test with an artificial *H. contortus* infection described later. Resistant sire candidates were selected based primarily on the mean FEC and mean packed cell volume monitored after the artificial infection. Resistant females of different ages were selected based on the multiple gastrointestinal nematode FEC and pack cell volume data collected on-farm. In the second and third year, progeny from the selected dams and sires with resistance to *H. contortus* from Langston University and the five commercial farms were tested in the central performance test. Sheep and goats were grouped per breed and species in adjacent pens with automated feeders allowing free-choice access to a 15% crude protein diet at Langston University.

Deworming and *H. contortus* artificial infection methods are described in a previous publication (13). Briefly, sheep and goats were treated with albendazole (Valbazen®; 10 and 20 mg per kg of body weight, respectively) and levamisole (Prohibit®; 12 and 18 mg per kg of body weight, respectively) during 2 weeks of adaptation. Then, animals were screened for FEC reduction (< 600 epg), and received an oral dose of 10,000 L3 larvae of *H. contortus*. FEC was recorded at 28, 35 and 42 days post-infection. Phenotypic records related to FEC and other traits (average daily gain, packed cell volume, IgA, IgG and IgM levels) evaluated in sheep and goats have been previously reported (13).

Genotyping and data quality control

DNA samples from sheep and goats were isolated from blood samples collected from the jugular vein in EDTA vacutainer tubes. Two hundred and fifty ng/μL of genomic DNA was genotyped using Capture-Seq by RAPiD Genomics (Gainesville, Florida) to target 100 genes related to the immune response against *H. contortus* or other GNI. The Oar_v4.0 reference genome (47) was used to design biotinylated 120-mer probes that captured sequences at each target locus. Then, samples were sequenced using next generation sequencing and sequencing data was demultiplexed, cleaned, and trimmed. The 3’ ends were trimmed by removing low quality bases with < 20 quality score reads. Clean reads were aligned to genome with MOSAIK software (48). Freebayes was used for identification of SNPs and VCFtools (49) was used to generate VCF files. SNPs with a call rate < 95% and MAF $\leq$ 0.01 were removed.

In order to visualize the genetic relationship between individuals and cluster the breeds under study, heatmaps were constructed using R software (50). Principal component analysis plots were generated to illustrate population structure using JMP Genomics 9 software from SAS (SAS Institute Inc., Cary, NC).

Signatures of selection using *F*st statistic per SNP

Signatures of selection were identified using *F*st statistic per SNP. This approach focuses on the identification of differences in allele frequencies between subpopulations. To identify genetic divergence between subpopulations, analyses between resistant and susceptible breeds within species were carried out. St. Croix and Katahdin were considered resistant sheep breeds and were compared against Dorper which was considered the susceptible sheep breed. Similarly, for goats, Spanish and Kiko breeds were classified as resistant and compared against the susceptible Boer breed. To identify any signatures of selection different in the two resistant breeds, an additional analysis was performed by comparing the St. Croix against Katahdin for sheep, and Spanish against Kiko for goats.

Calculation of average allele frequency across breeds, estimation of total variance, and deviation of each population from mean and *F*st computation were performed using the R software and the R codes from Gondro et al. (9). The formula used to estimate *F*st values was the following:

where σ^2subpopulation is the variance of the deviation of each population from mean and σ^2total is the total variance. Estimates corresponding to the 1% extreme *F*st values were used to define a significance threshold and identify regions under selection. Candidate SNP under selection were identified using the Oar_v4.0 reference genome from NCBI. The Online Mendelian Inheritance in Man website and scientific literature were used to determine gene function. Cytoscape v3.7.1 software was used for gene network graphics (51).

Abbreviations

FEC: Fecal egg count

GNI: Gastrointestinal nematode infections

OAR: Chromosome number

Declarations

Ethics approval and consent to participate

The research protocol for the present study was approved by the Langston University Animal Care and Use Committee.

Consent for publication

Not applicable.

Availability of data and materials

The datasets generated during and/or analyzed during the current study are available in the [Ensembl] repository (submitted, waiting for approval).

Competing Interest

The authors declare that they have no competing interests.

Funding

This study was supported by UF Agricultural Station and the National Institute of Food and Agriculture, U.S. Department of Agriculture, under award No. 003673. The project was supported by the USDA National Institute of Food and Agriculture 1890 Institution Capacity Building Grant Program, Project OKLXSAHLU12, Accession Number 230541. Z.M. Estrada- Reyes was partially supported by the Consejo Nacional de Ciencias y Tecnología, CONACYT.

Authors' contributions

Estrada-Reyes conducted all analyses of the samples evaluates and wrote the drafted manuscript. Mateescu R conceived and assisted with the analysis and manuscript. Tsukahara Y, Goetsch AL, Gipson TA, Sahlu T, Puchala R, Wang Z and Hart SP assisted with the manuscript, collected the FEC data, and selected the animals with low FEC.

Acknowledgements

We gratefully acknowledge Dr. Francisco Peñagaricano, Assistant Professor at the University of Florida and Dr. Rostam Abdollahi-Arpanahi, Postdoctoral Researcher at the University of Florida, USA, and Assistant Professor at the University of Tehran, Iran, for their guidance.

References

1. USDA-APHIS-VS. 2017. Goat and Kid Predator and Nonpredator Death Loss in the United States, 2015. USDA–APHIS–VS–CEAH, National Animal Health Monitoring System, Fort Collins, CO.

2. USDA-APHIS-VS. 2015. Sheep and Lamb Predator and Nonpredator Death Loss in the United States. USDA–APHIS–VS–CEAH, National Animal Health Monitoring System, Fort Collins, CO.

3. Andersson L. How selective sweeps in domestic animals provide new insight into biological mechanisms. J. Intern. Med. 2012; doi:10.1111/j.1365-2796.2011.02450.x.

4. Zvinorova PI., Halimani TE, Muchadeyi FC, Matika O, Riggio V, and Dzama K. Breeding for resistance to gastrointestinal nematodes - the potential in low-input/output small ruminant production systems. Vet

Parasitol. 2016; doi:10.1016/j.vetpar.2016.05.015.

5. Bisset SA, Morris CA, McEwan JC and Vlassoff A. Breeding sheep in New Zealand that are less reliant on anthelmintics to maintain health and productivity. NZ Vet. J. 2001; 49.

6. Eady SJ, Woolaston RR, Mortimer SI, Lewer RP, Raadsma HW. Resistance to nematode parasites in Merino sheep: sources of genetic variation. Aust. J. Agric. Res. 1996; 47.

7. McEwan JC, Dodds KG, Greer GJ, et al. Genetic estimates for parasite resistance traits in sheep and their correlations with production traits. NZ J. Zool. 1995; 22: 177.

8. Chessa B, Pereira F, Arnaud F, Amorim A, Goyache F, Mainland I, et al. Revealing the history of sheep domestication using retrovirus integrations. Science 2009; doi:10.1126/science.1170587.

9. Gondro C, van der Werf J, and Hayes B. In: Genome-Wide Association Studies and Genomic Prediction, Methods in Molecular Biology. Springer Science Business Media, LLC. 2013. p. 423-436. doi: 10.1007/978-1-62703-447-0_19.

10. Purfield DC, McParland S, Wall E, and Berry DP. The distribution of runs of homozygosity and selection signatures in six commercial meat sheep breeds. PLoS One. 2017; doi.org:10.1371/journal.pone.0176780.

11. McRae KM, McEwan JC, Dodds KG, and Gemmell NJ. Signatures of selection in sheep bred for resistance or susceptibility to gastrointestinal nematodes. BMC Genomics. 2014; doi: 10.1186/1471-2164-15-637.

12. Onzima RB, Upadhyay MR, Doekes HP, et al. Genome-Wide Characterization of Selection Signatures and Runs of Homozygosity in Ugandan Goat Breeds. Front Genet. 2018; 9:318. doi:10.3389/fgene.2018.00318.

13. Estrada-Reyes, Z. M., Tsukahara, Y., Goetsch, A. L., Gipson, T. A., Sahlu, T., Puchala, R., et al. Effect of Ovar-DRA and Ovar-DRB1 genotype in small ruminants with haemonchosis. Parasite Immunol. 2018; doi.org:10.1111/pim.12534.

14. Kemper KE, Emery DL, Bishop SC, Oddy H, Hayes BJ, Dominik S, et al. The distribution of SNP marker effects for faecal worm egg count in sheep, and the feasibility of using these markers to predict genetic merit for resistance to worm infections. Genet Res. 2011; doi: 10.1017/S0016672311000097.

15. Benavides MV, Sonstegard TS, Kemp S, Mugambi JM, Gibson JP, Baker RL, et al. Identification of novel loci associated with gastrointestinal parasite resistance in a red Maasai × Dorper backcross population. PLoS One. 2015;.doi:10.1371/journal.pone.0122797.

16. Silva, FF, Bambou JC, Oliveira JA, Barbier C, Fleury J, Machado T, et al. Genome wide association study reveals new candidate genes for resistance to nematodes in Creole goat. Small Rumin. Res. 2018;

doi:10.1016/j.smallrumres.2018.06.004.

17. Starr R, Willson TA, Viney EM, Murray LJ, Rayner JR, Jenkins BJ, et al. A family of cytokine-inducible inhibitors of signaling. Nature. 1997; doi: 10.1038/43206.

18. Esper L, Roman-Campos D, Lara A, Brant F, Castro LL, Barroso A, et al. Role of SOCS2 in Modulating Heart Damage and Function in a Murine Model of Acute Chagas Disease. Am. J. of Pathol. 2012; doi.org:10.1016/j.ajpath.2012.03.042.

19. Knosp CA, Carroll HP, Elliott J, Saunders SP, Nel HJ, Amu S, et al. SOCS2 regulates T helper type 2 differentiation and the generation of type 2 allergic responses. J. Exp. Med. 2011; doi: 10.1084/jem.20101167 PMID: 21646394.

20. Metcalf D, Greenhalgh CJ, Viney E, Willson TA, Starr R, Nicola NA, et al. Gigantism in mice lacking suppressor of cytokine signalling-2. Nature. 2000; doi.org:10.1038/35016611.

21. Gossner A, Wilkie H, Joshi A, and Hopkins J. Exploring the abomasal lymph node transcriptome for genes associated with resistance to the sheep nematode Teladorsagia circumcincta. Vet. Res. 2013; doi: 10.1186/1297-9716-44-68 PMID: 23927007.

22. Nde P, Lima M, Johnson C, Pratap S, and Villalta F. Regulation and use of the extracellular matrix by Trypanosoma cruzi during early infection. Front. Immunol. 2012; doi:10.3389/fimmu.2012.00337.

23. Araujo, R. N., Padilha, T., Zarlenga, D., Sonstegard, T., Connor, E. E., Van Tassel, C., et al. Use of a candidate gene array to delineate gene expression patterns in cattle selected for resistance or susceptibility to intestinal nematodes. Vet. Parasitol. 2009; doi:10.1016/j.vetpar.2008.12.017.

24. Robinson N, Pleasance J, Piedrafita D, Meeusen EN. The kinetics of local cytokine and galectin expression after challenge infection with the gastrointestinal nematode, Haemonchus contortus. Int. J. Parasitol. 2011; doi:10.1016/j.ijpara.2010.11.006.

25. Oshiro TM, Macedo MS, and Macedo-Soares MF. Anti-inflammatory activity of PAS-1, a protein component of Ascaris suum. Inflamm. Res. 2005; doi: 10.1007/s00011-004-1316-7.

26. Maizels RM, and Yazdanbakhsh M. Immune Regulation by helminth parasites: cellular and molecular mechanisms. Nat. Rev. Immunol. 2003; doi.org:10.1038/nri1183.

27. Ingham A, Reverter A, Windon R, Hunt P, and Menzies M. Gastrointestinal nematode challenge induces some conserved gene expression changes in the gut mucosa of genetically resistant sheep. Int. J. Parasitol. 2008; doi:10.1016/j.ijpara.2007.07.012.

28. Thomas PG, Carter MR, Atochina O, Akram AD, Piskorska D, McGuire E, and Harn D. Maturation of dendritic cell 2 phenotype by a helminth glycan uses a toll-like receptor 4-dependent mechanism. J Immunol. 2003; doi: 10.4049/jimmunol.171.11.5837.

29. Maizels RM, and McSorley HJ. Regulation of the host immune system by helminth parasites. J. Allergy Clin. Immunol. 2016; doi:10.1016/j.jaci.2016.07.007.

30. Hassan M, Hanrahan JP, Good B, Mulcahy G, and Sweeney T. A differential interplay between the expression of Th1/Th2/Treg related cytokine genes in Teladorsagia circumcincta infected DRB1*1101 carrier lambs. Vet. Res. 2011; doi: 10.1186/1297-9716-42-45.

31. Bell BD, Kitajima M, Larson RP, et al. The transcription factor STAT5 is critical in dendritic cells for the development of TH2 but not TH1 responses. Nat Immunol. 2013; 14(4):364-371. doi:10.1038/ni.2541.

32. Taylor DK, Walsh PT, LaRosa DF, Zhang J, Burchil MA, Farrar MA. and Turka LA. Constitutive activation of stat5 supersedes the requirement for cytokine and tcr engagement of cd4+ t cells in steady-state homeostasis. J Immunol. 2006; doi: 10.4049/jimmunol.177.4.2216.

33. Ahmed AM, Good B, Hanrahan B, McGettigan JP, Browne P, Keane J, et al.. Variation in the Ovine Abomasal Lymph Node Transcriptome between Breeds Known to Differ in Resistance to the Gastrointestinal Nematode. PLoS One. 2015; doi:10.1371/journal.pone.0124823.

34. Jallow OA, McGregor BA, Anderson N, and Holmes JHG. Intake of trichostrongylid larvae by goats and sheep grazing together. Aust. Vet. J. 1994; doi.org:10.1111/j.1751-0813.1994.tb00927.x.

35. Cayrol C, and Girard JP. IL-33: an alarmin cytokine with crucial roles in innate immunity, inflammation and allergy. Curr. Opin. Immunol. 2014; doi:https://doi.org/10.1016/j.coi.2014.09.004.

36. Rank MA, Kobayashi T, Kozaki H, Bartemes KR, Squillace DL, and Kita H. IL-33–activated dendritic cells induce an atypical TH2-type response. J. Allergy Clin. Immunol. 2009; doi:https://doi.org/10.1016/j.jaci.2009.02.026.

37. Bancroft AJ, Else KJ, Sypek JP, and Grencis RK. Interleukin-12 promotes a chronic intestinal nematode infection. Eur. J. Immunol. 1997; doi: 10.1002/eji.1830270410.

38. Rotman HL, Schnyder CS, Scott P, Nolan TJ, Schad GA, and Abraham D. IL-12 eliminates the Th-2 dependent protective immune response of mice to larval Strongyloides stercoralis. Parasite Immunol. 1997; doi: 10.1046/j.1365-3024.1997.d01-142.x.

39. Venturina VM, Gossner AG, and Hopkins J. The immunology and genetics of resistance of sheep to Teladorsagia circumcincta. Vet. Res. Commun. 2013; doi: 10.1007/s11259-013-9559-9.

40. Hoste H., Torres-Acosta JFJ, and Aguilar-Caballero AJ. Nutrition-parasite interactions in goats: Is immunoregulation involved in the control of gastrointestinal nematodes? Parasite Immunol.2008; doi:10.1111/j.1365-3024.2007.00987.x

41. Pernthaner A, Cole SA, Morrison L, and Hein WR. Increased expression of interleukin-5 (IL-5), IL-13, and tumor necrosis factor alpha genes in intestinal lymph cells of sheep selected for enhanced resistance to

nematodes during infection with Trichostrongylus colubriformis. Infect Immunol. 2005; doi:10.1128/IAI.73.4.2175-2183.2005.

42. Patterson DM, Jackson F, Huntley JF, Stevenson LM, Jones DG, Jackson E, et al. Studies on caprine responsiveness to nematodiasis: segregation of male goats into responders and non-responders. Int. J. Parasitol. 1996a; doi: 10.1016/0020-7519(95)00121-2.

43. Patterson DM, Jackson F, Huntley JF, Stevenson LM, Jones DG, Jackson E, et al. The response of breeding does to nematodiasis: segregation into ‘responders’ and ‘non-responders’. Int. J. Parasitol. 1996b; doi: 10.1016/S0020-7519(96)00125-7.

44. Etter E, Hoste H, Chartier C, et al. Susceptibility of high and low producer dairy goats to single experimental infection with Trichostrongylus colubriformis. Parasitol. Res. 2000; doi.org:10.1007/s004360000280.

45. Meeusen EN, Balic A and Bowles VM. Cells, cytokines and other molecules associated with rejection of gastrointestinal nematode parasites. Vet Immunol Immunopathol. 2005; doi:10.1016/j.vetimm.2005.07.002.

46. Alba-Hurtado F, and Muñoz-Guzmán MA. Immune responses associated with resistance to haemonchosis in sheep. Biomed. Res. Int. 2013; doi:10.1155/2013/162158

47. http://www.sheephapmap.org/

48. Lee W, Stromberg MP, Ward A, Stewart C, Garrison EP, Marth GT. MOSAIK: A Hash-Based Algorithm for Accurate Next- Generation Sequencing Short-Read Mapping. 2014; 9(3). doi:10.1371/journal.pone.0090581.

49. https://vcftools.github.io/

50. https://www.r-project.org/

51. Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, Amin N, Schwikowski B, Ideker T. Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res. 2003; 11:2498-2504.

Figures

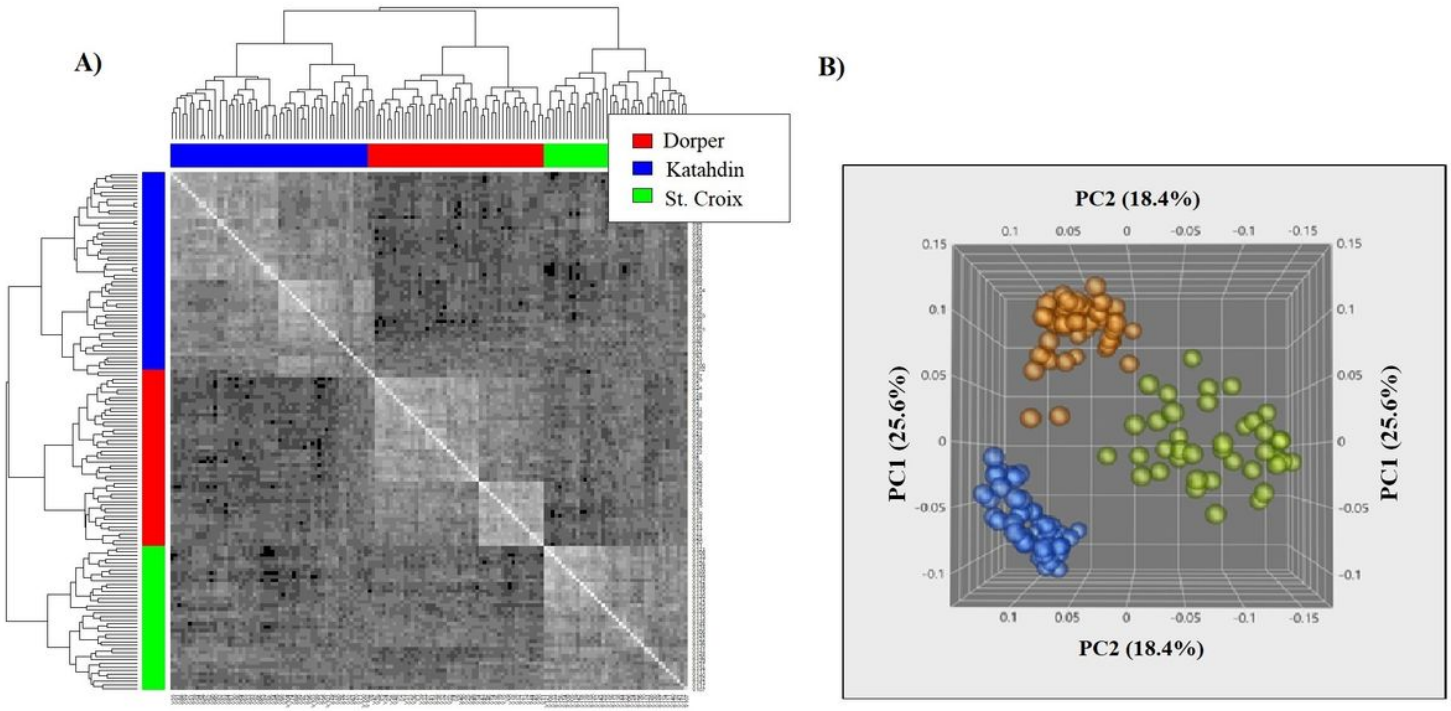

Figure 1

Clustering of sheep based on the genetic relationships. Animals were colored based on their known breed: Dorper (blue), Katahdin (red) and St. Croix (green) sheep breeds. One cluster per breed was identified on the heatmap (A). The PCA plot (B) shows the population structure of the breeds under the study based on the first two principal components (PC). The PC1 and PC2 explain 25.6 % and 18.4% of the total variation observed in the sheep populations, respectively.

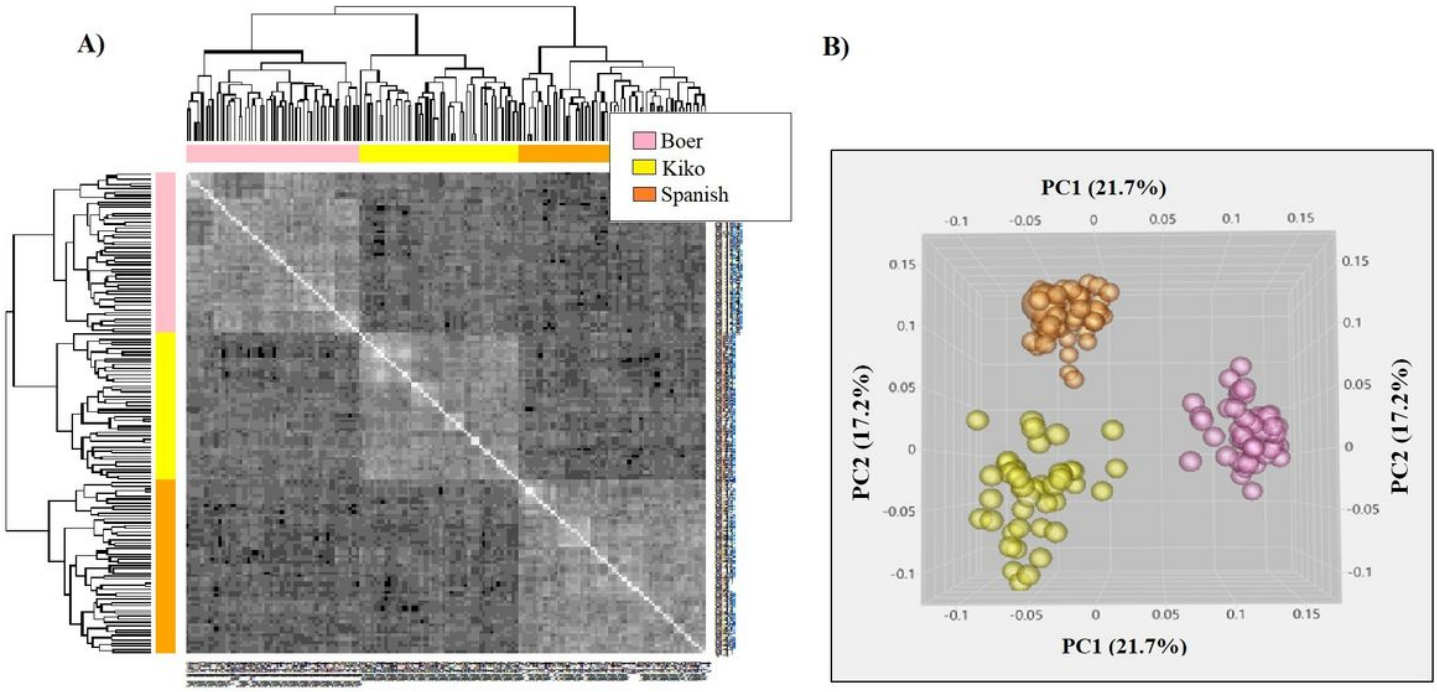

Figure 2

Clustering of goats based on the genetic relationships. Animals were colored based on their known breed: Boer (pink), Kiko (yellow) and Spanish (orange) goat breeds. One cluster per breed was identified on the heatmap (A). The PCA plot (B) shows the population structure of the breeds under the study based on the first two principal components (PC). The PC1 and PC2 explain 21.7 % and 17.2% of the total variation observed in the sheep populations, respectively.

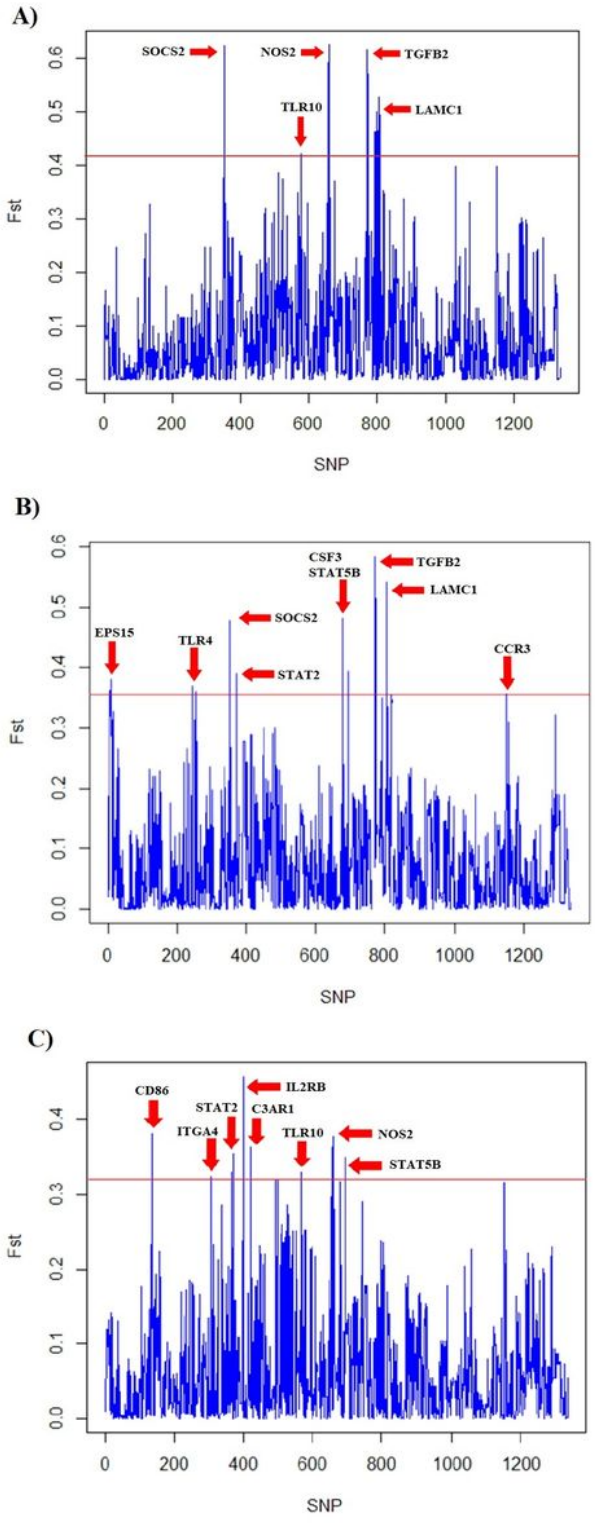

Figure 3

Graphical representation of the Fst values per SNP from targeted genomic regions in sheep. The SNP data is ordered based on chromosomal location (x-axis). Red arrows indicate the genes with SNP loci under selection between Katahdin vs Dorper (a), St. Croix vs Dorper (b), and St. Croix vs Katahdin (c) sheep.

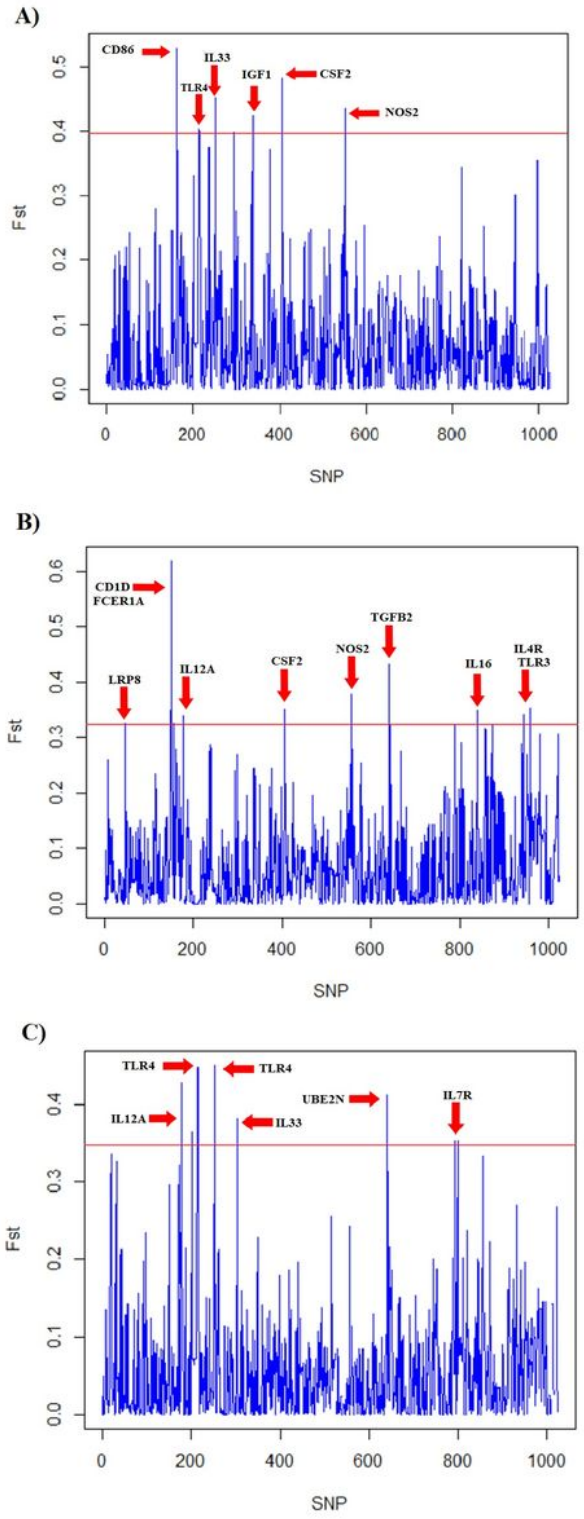

Figure 4

Graphical representation of the Fst values per SNP from targeted genomic regions in sheep. The SNP data is ordered based on chromosomal location (x-axis). Red arrows indicate the genes with SNP loci under selection between Kiko vs Boer (a), Spanish vs Boer (b), and Spanish vs Kiko (c) goats.

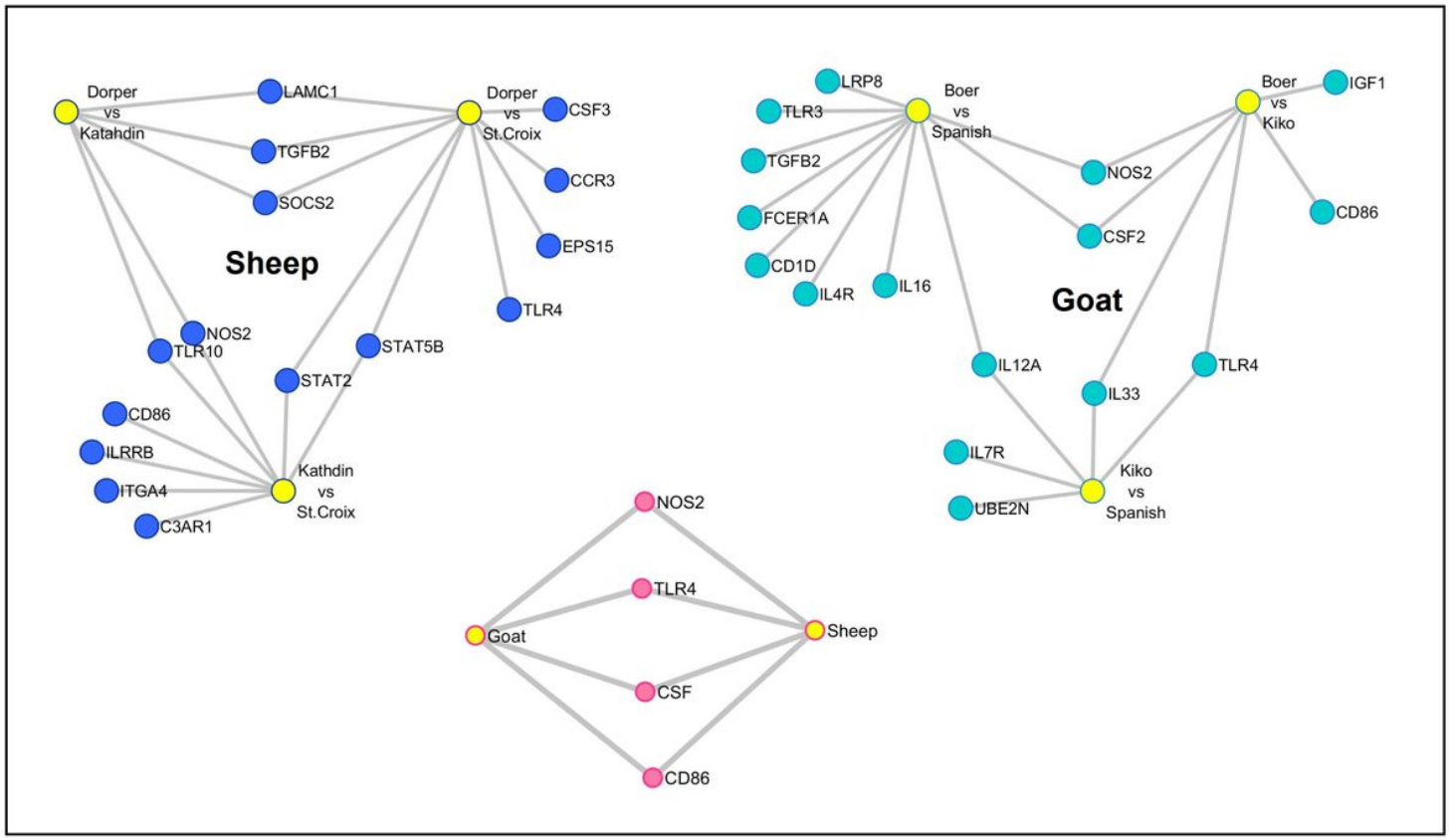

Figure 5

Network of genes observed under selection in sheep (dark blue) and goat (light blue) populations. The yellow dots represent the two populations compared in the Fst analysis. The green dots represent the genes under selection in both species.