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Expression of the DRB1*1101 Allele in Meat Goats Pasture Exposed to *Haemonchus contortus*

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Abstract

The Major Histocompatibility class II complex plays an extremely important role in disease resistance and susceptibility. A mutation in the DRB1*1101 gene of the MHC II has been implicated in parasite resistance in sheep. Evaluation of the DRB1*1101 allele and the role it plays in *Haemonchus contortus* infected goats has not been addressed. This study evaluated expression of the DRB1*1101 allele in goats naturally infected with *Haemonchus contortus* infection. Total RNA was isolated from whole blood and RT-PCR (Reverse-Transcriptase Polymerase Chain Reaction) using primers designed to target the conserved regions of the human and sheep DRB1*1101 allele. The expected cDNA product was observed at the 201bp of the targeted DRB1*1101 region and verified by cloning and sequencing. Alignment of the Goat DRB1*1101 sequence showed 92% homology at the transcriptional level and 86% homology at the translational level with sheep. Goats pasture exposed to *Haemonchus contortus* had elevated ($P < 0.05$) DRB1*1101 allele expression. Expression was highest ($P < 0.05$) in Boer goats, lower in Myotonic goats and lowest ($P < 0.05$) in Spanish goats. Males had higher ($P < 0.05$) DRB1*1101 expression than females. DRB1*1101 gene expression was higher ($P < 0.05$) in younger vs. older goats. Goats showing resistance to *Haemonchus contortus* had lower ($P < 0.05$) DRB1*1101 expression than susceptible goats. Based on our findings, the DRB1*1101 gene in goats can potentially be used as a genetic marker for either resistance or susceptibility to *Haemonchus contortus*.

Keywords: *Haemonchus contortus*, gene expression, DRB1*1101, goat

1. Introduction

Gastrointestinal nematode (GIN) infection is a major problem in the small ruminant industry worldwide. According to southern states in Northern America, sixty-two percent of small ruminant producers have problems with GIN. The GIN that causes the most economic burden in small ruminants, are within the Order Strongylida (Keane et al., 2006). A major GIN of concern is *Haemonchus contortus*. *Haemonchus contortus* is a blood sucking nematode that causes severe blood loss which can lead to anemia, anorexia, depression, loss of condition, and eventual death (Clark, Keisel, & Goby, 1962; Le Jambre, 1995; Rowe, Nolan, de Chaneet, Teleni, & Holmes, 1988). *Haemonchus contortus* costs the global small ruminant industry billions of dollars a year spent on anthelmintics and other treatments of this GIN. The major histocompatibility complex (MHC) is a cell surface molecule involved in antigen presentation by immune cells (B lymphocytes, Dendritic cells, and Macrophages). The MHC class II mediates specific immunity, to an antigen. The primary function of the MHC complex is to code for histocompatibility molecules that are antigen presenting receptor glycoproteins (Dukkipati, Blair, Garrick, & Murray, 2006). In ruminants, DRB1*1101 falls in the class MHC IIa (Andersson & Rask, 1988). The DRB is the most polymorphic locus of the MHC gene complex (Andersson & Rask, 1988). The DR genes are highly polymorphic and the molecules encoded by these genes are highly expressed on the cell membranes of macrophages and B cells (Outteridge et al., 1996). Because of the association between the MHC complex and the resistance to nematodes there have been studies on the MHC complex in many mammals (Axtner & Sommer, 2012; Paterson, Wilson, & Pemberton, 1998; Sayers et al., 2005; Schwaiger et al., 1995; Stear, Innocent, & Buitkamp, 2005; Buítkamp, Filmether, Stear, & Epplen, 1996; Stear et al., 1996; Stear et al., 2007). The DRB1*1101 exon 2 encodes the β1 domain, which makes up part of the PBR (peptide binding region) of the DR molecules. It has been found that the residues found in this region have close contact with the peptides that are presented in the PBR or the TCR (T-cell receptor) region (Brown et al., 1993, 2015). Thus this region(s) is likely

to have functions such as disease resistance and susceptibility (Buítkamp et al., 1996; Li et al., 2011; Sayers et al., 2005; Schwaiger et al., 1995; Untalan, Pruett, & Steelman, 2007; Valilou et al., 2015). Sheep carrying the DRB1*1101 in the second exon has shown resistance to the GIN *Teladorsagia circumcincta* (Hassan et al., 2011). Studies on the evaluation of the DRB1*1101 exon 2 in goats pasture exposed to *Haemonchus contortus* have not been conducted. Therefore the objective of this study was to isolate and characterize expression of the DRB1*1101 (Exon 2) allele in meat goats grazing *Haemonchus contortus* infected pastures.

2. Materials and Methods

2.1 Experimental Animals and Haemonchus contortus Verification

Meat goats (Boer, Spanish and Myotonic) ranging in ages from 1-5 years old, grazing pasture and supplemented with hay, cracked corn (78%) and ground soybean meal (20%) were housed at VSU Randolph farm in accordance with institutional animal care and use guidelines. Records were kept on these goats as to their dates of birth, place of origin etc.). More than 100 Goats were screened for parasite load and clinical anemia status via fecal egg counts (FEC) in eggs per gram (EPG), packed cell volume (PCV) and FAMACHA eye color charts (FAM), and divided into susceptible and resistant groups accordingly. Our previously published work describe these procedures in detail (Corley & Jarmon, 2012b). The FEC, FAM and PCV data collected were analyzed using SAS version 9.3, (Cary, North Carolina). We determined that goats with > 2000 EPG with PCV $\leq$ 18 were naturally susceptible and those goats with > 2000 FEC with PCV $\geq$ 18 were resistant. From the resistant and susceptible groups, thirty animals were sacrificed and blood samples collected and stored at -80 ^{o}C for molecular analysis. *Haemonchus contortus* spp. was verified via nucleotide sequencing as previously published (Corley & Jarmon, 2012a). Nucleotide sequences were analyzed using sequence analysis software (NCBI-BLAST (Altschul, Gish, Miller, Myers, & Lipman, 1990), CLC Main Workbench).

2.2 Goat Blood Collection and Preparation

Goats were adequately restrained and blood collected via jugular venipuncture. In brief, the vein was identified by palpation and visual inspection. The area was swabbed with 70% alcohol. Gentle pressure was applied at the thoracic inlet to produce distension of the vein. Blood samples (3 ml) were collected in vials without coagulant using 16-20G needles. Blood samples were subsequently stored at -80 °C for later molecular (RNA) analysis.

2.3 Total RNA Extraction from Goat Blood

Total RNA was isolated from selected goat whole blood samples previously stored at -80 °C using the modified RNA extraction protocol (Gauthier, Madison, & Michel, 1997). Approximately 500 µl of each sample was mixed with 3 ml of 1X Dulbecco's phosphate buffered saline (Invitrogen; Grand Island, NY 14072) and centrifuged at 5,000 rpm for 20 minutes at 4 °C in a Heraeus MegaFuge 16R centrifuge (ThermoFisher-Scientific). After discarding the supernatant, the addition of PBS and centrifugation was repeated until cells were clean. Once the cells were adequately washed, 1 ml of Guanidium Thiocynate (4 M GTC, 25 mM Sodium citrate pH 7.0 and N-0.5% lauroylsarcosine, Sigma-Aldrich) was added and the cells were suspended by pipetting. Under the fume hood, 10 µl of β-mercaptoethanol (Omni-Pur), 2M sodium acetate (pH4) at one-tenth of the volume and 1 volume of phenol:chloroform:isoamyl alcohol (25:24:1) (Fisher Scientific; Fair Lawn, New Jersey 0740) were added and mixed before placing on ice for 15 minutes. The tubes were then placed in the centrifuge for 15 minutes at 4 °C and 5,000 rpm. After centrifugation, the aqueous layer was removed and placed into a sterile tube. The samples were placed on ice for 10 minutes after the addition of 2 volumes of 95% ethanol before being placed in the centrifuge for 15 minutes at 5,000 rpm, allowing RNA to precipitate. Two additional washes with 15 minutes of centrifugation at 5,000 rpm were completed using 200 µl of 70% ethanol before allowing samples to air dry. Samples were then resuspended in Diethylpyrocarbonate (DEPC) treated water and stored at -80 °C. A NanoDrop 2000 Spectrophotometer (ThermoScientific) was used to measure RNA concentration and purity.

*2.4 Reverse Transcriptase PCR (RT-PCR) of Goat DRB1*1101*

Oligonucleotide primers were designed from mRNA of the sheep DRB1*1101 nucleotide sequences using the bioinformatics software, CLC Main Workbench (http://www.clcbio.com). Primers and target regions used for isolation of the goat DRB1*1101 gene are given in Table 1. The RT-PCR was conducted using the recommended protocol of the Verso 1-step RT-PCR kit (Thermo Scientific). Modified thermocyling conditions for 40 cycles were as follows: 50 °C 15 minutes, 95 °C, 2 minutes (initial denaturation), 95 °C, 30 secs, 55 °C, 1 minute, 72 °C, 1 minute repeated 39 times and a final extension at 72 °C for 5 minutes. The target DRB1*1101 cDNA was visualized by 1.5% agarose gel electrophoresis and a UGenius UV gel documentation system (SynGene, Fredericksburg, MD) equipped with a high resolution CCD camera.

Table1. Primers and Target Regions used for Isolation of the goat DRB1*1101 gene from goat blood

Primer name	Conserved Primer sequence	Target Region	Fragment Length (bp)
Forward	5'- TCTTCAATGGGACGGAGC -3'	37-54	201
Reverse	5'- CGTAGTTGTGTCTGCAGT -3'	220-237 (Rev-comp)	

*2.5 Nucleotide Sequencing of Goat DRB1*1101 cDNA*

For DRB1*1101 nucleotide sequencing, the cDNA (201 bp) products were cut out, purified from agarose gels (Qiagen and Bio-Rad) and cloned using the TOPO TA vector (Life Technologies) and transformed in *E.coli* DH5 alpha. Colonies were screened (Blue, White), cDNA inserts verified via PCR (DRB1*1101 primers) and positives prepared for nucleotide sequencing per commercial instructions. Samples were sent for sequencing at GeneWiz (South Plainfield, New Jersey). Raw nucleotide sequences were analyzed using sequence analysis software (NCBI-BLAST, CLC Main Workbench). Subsequently, Gene Tools software (SynGene, Fredericksburg, MD) analysis was conducted to determine gene expression of goat DRB1*1101 cDNA.

*2.6 Measurement of Goat DRB1*1101 Gene Expression*

Gene expression of DRB1*1101 in goat blood was measured via UV Gel Documentation and GeneTools analytical software. Area densities of each sample were measured and standardized with automatic correction for background. An average of three replications was measured. The analytical parameters were as previously published (Corley & Jarmon, 2012a).

3. Statistical Analysis

Area density quantitation data were analyzed using the General Linear Model procedure of SAS and Pearson Correlation analysis. Means (n = 30) were considered significant at $P < 0.05$. Breed, age and gender differences were considered.

4. Results and Discussion

Partial codons of the Goat DRB1*1101 exon 2 were successfully isolated. The expected 201 bp product was observed, purified, cloned and sequenced (Figure 1). Sequence alignment of the goat with sheep DRB1*1101 showed 92% homology at the transcriptional level (Figure 2) and 86% homology at the translational level (Figure 3). This sequence has been submitted to the NCBI GenBank (Accession No. KM196595). Expression of the DRB1*1101 was elevated ($P < 0.05$) in different breeds of meat goats pasture exposed to *Haemonchus contortus*. Gene expression was highest ($P < 0.05$) in Boer goats, lower in Myotonic goats and lowest ($P < 0.05$) in Spanish goats (Figure 4) when compared to each other. Gender differences ($P < 0.05$) in DRB1*1101 expression were observed (Figure 5) where males showed higher ($P < 0.05$) expression of the DRB1*1101 than females. These support previous studies that show male goats are more susceptible to *Haemonchus contortus* infection that females (Corley & Jarmon, 2012a, 2012b). Studies also show that male sheep exhibit more susceptibility to GIN than females, which may or may not be due to a testosterone effect (Saddiqi et al., 2011; Saddiqi, Sarwar, Iqbal, Nisa, & Shahzad, 2012). Age differences influenced DRB1*1101 gene expression. The DRB1*1101 allelic expression was higher ($P < 0.05$) in younger (4 yrs old) than older (> 4 yrs old) (Figure 6). These data would support the theory that younger goats would be more susceptible to *Haemonchus contortus* and not yet undergone much gene conversion at the DRB1 gene locus. Overall, regardless of breed, DRB1*1101 expression was higher ($P < 0.05$) in naturally susceptible ($\leq$ 18 PCV; > 2000 FEC) than in resistant ($\geq$ 18 PCV; > 2000 FEC) groups of pasture infected goats (Figure 7) when compared to each other. The packed cell volume was somewhat negatively (47%) correlated ($P < 0.05$) with DRB1*1101 expression, indicating that the DRB1*1101 was more correlated with susceptibility to *Haemonchus contortus* rather than resistance (data not shown). However, in the context of resistance or susceptibility to *Haemonchus contortus*, expression of the DRB1*1101 allele was different within breeds. Boer and Spanish goats naturally resistant to *Haemonchus contortus* had elevated expression of the DRB1*1101 than those that were susceptible (Figures 8 and 9), whereas Myotonic goats naturally susceptible to *Haemonchus contortus* showed elevated expression of the DRB1*1101 allele (Figure 10). The DRB is the most polymorphic locus of the MHC gene complex (Andersson & Rask, 1988) therefore, results in this study are not unexpected, as the MHC is highly polymorphic and interbreed differences at the DRB1 locus is expected to be different depending on the length and time of exposure, and the gene conversion events that can occur at the MHC locus. These data also support the existence of allelic differences at the DRB1 locus in sheep with relevance to GIN infection (Polat, Aida, Takeshima, Aniwashi, & Halik, 2015; Sayers et al., 2005; Schwaiger et al., 1995). Since it has been shown that the MHC complex and nematode

infection are linked (Axtner & Sommer, 2012; Paterson et al., 1998; Sayers et al., 2005; Schwaiger et al., 1995; Stear et al., 2005), these data support that a correlation between DRB1*1101 expression and *Haemonchus contortus* infection in goats does exist. However due to the allelic diversity, a SNP approach looking for correlations between DRB1*1101 exon 2 and *Haemonchus contortus* in inter and intra breed experiments in goats is necessary. Isolation and characterization of the DRB1*1101 allele allows further elucidation of the mechanism of immunity to *Haemonchus contortus* infection in goats and in the future can lead to support innovative technology in the fight against damage to the small ruminant industry by *Haemonchus contortus*.

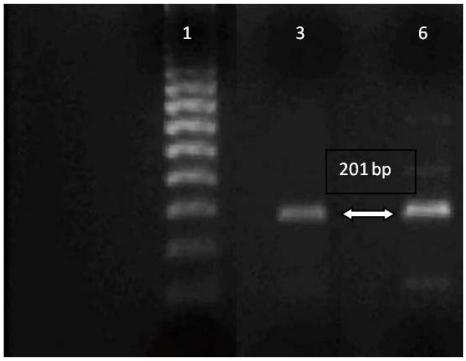

Figure 1. Agarose Gel Electrophoresis of Goat DRB1*1101 cDNA after Reverse Transcriptase PCR

Note. Lane 1 = Molecular Weight Marker, Lanes 3, 6 = Goat DRB1*1101 cDNA (201 bp).

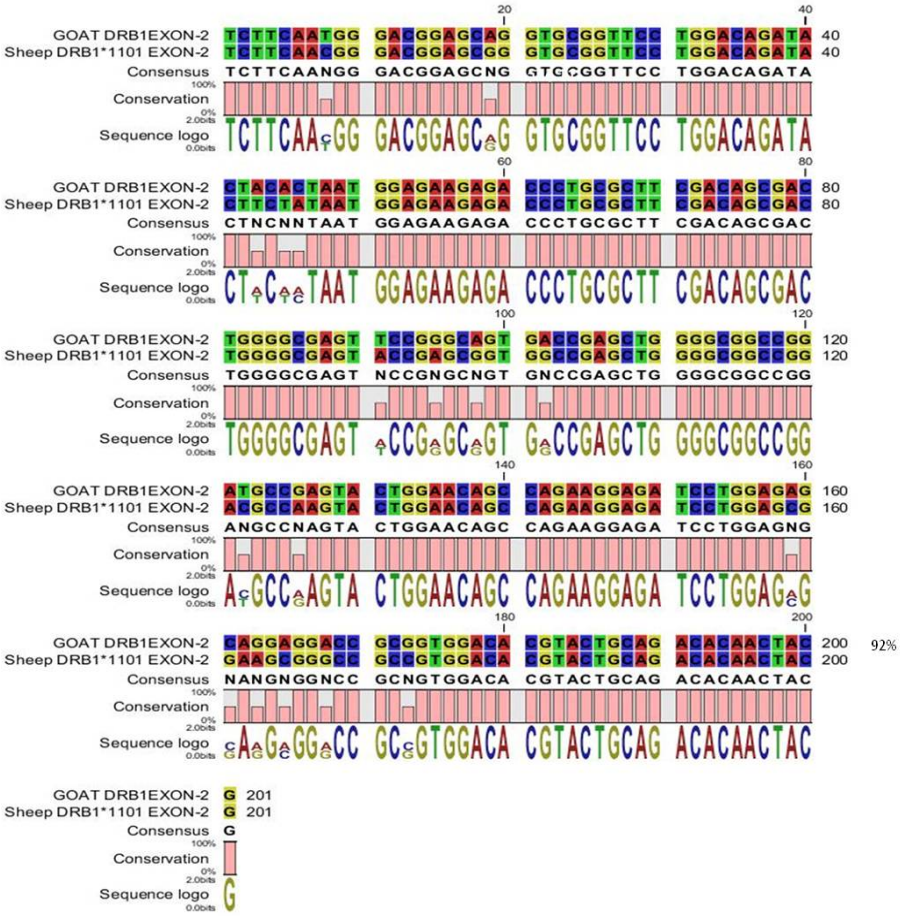

Figure 2. Nucleotide Sequence Alignment Showing Homology of Goat and Sheep DRB1*1101

Note. Generated using CLC Bioinformatic Software.

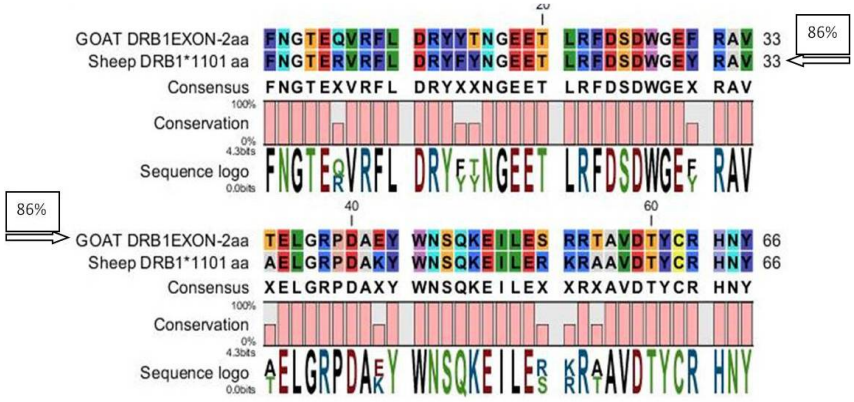

Figure 3. Amino Acid Alignment of Translated Goat and Sheep DRB1*1101

Note. Generated using CLC Bioinformatic Software.

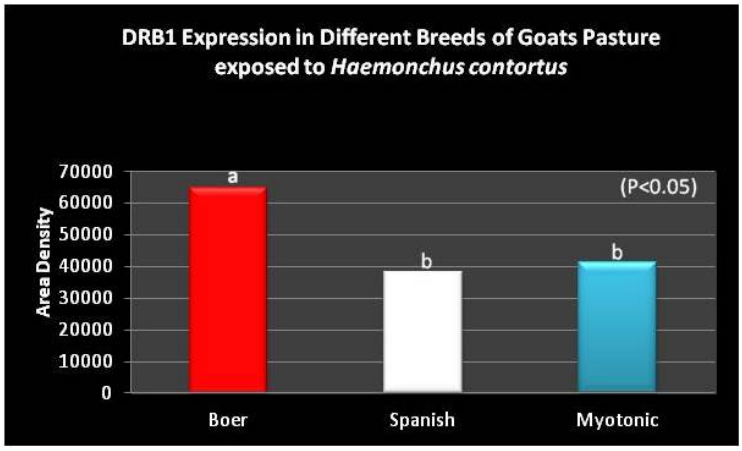

Figure 4. DRB1*1101 Expression in Different Breeds of Goats Pasture exposed to *Haemonchus contortus*

Note. ab, Means with different letters differ ($P < 0.05$).

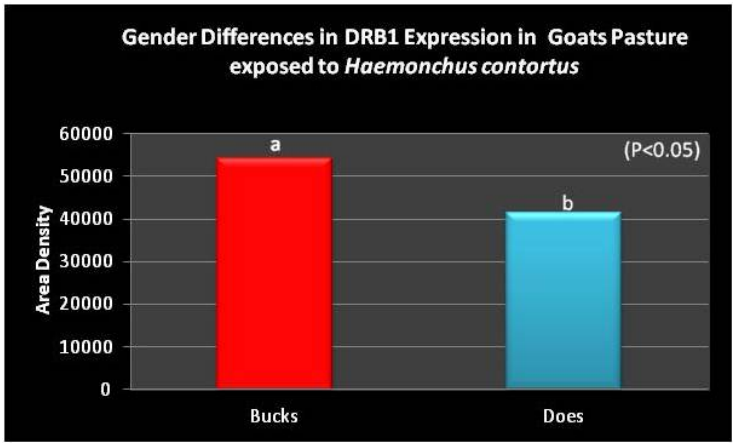

Figure 5. Gender Differences in DRB1*1101 Expression in Goats Pasture exposed to *Haemonchus contortus*

Note. ab, Means with different letters differ ($P < 0.05$).

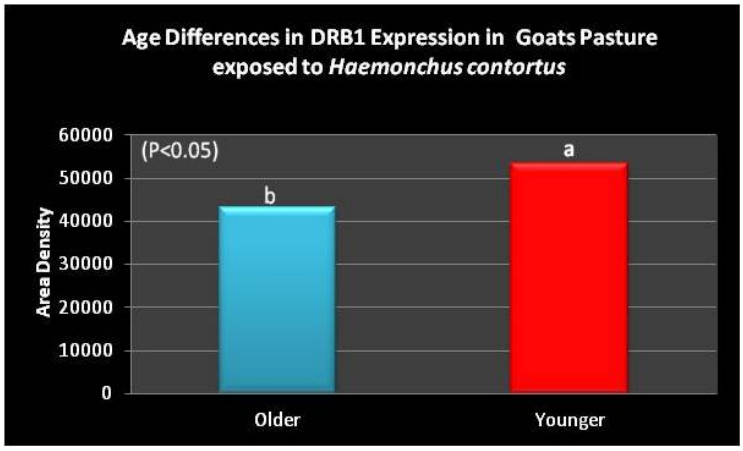

Figure 6. Age Differences in DRB1*1101 Expression in Goats Pasture exposed to *Haemonchus contortus*

Note. ab, Means with different letters differ ($P < 0.05$).

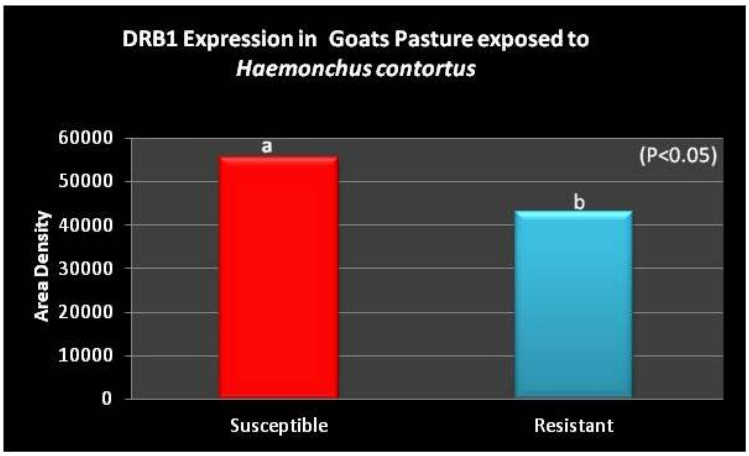

Figure 7. DRB1*1101 Expression in Goats Pasture exposed to *Haemonchus contortus*

Note. ab, Means with different letters differ ($P < 0.05$).

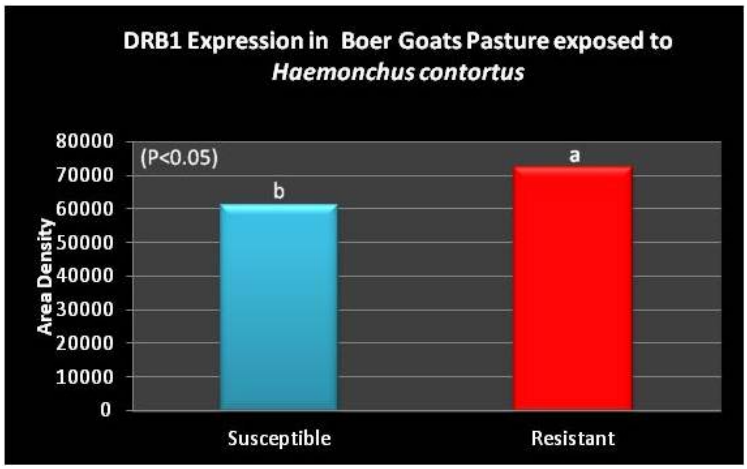

Figure 8. DRB1*1101 Expression in Boer Goats Pasture exposed to *Haemonchus contortus*

Note. ab, Means with different letters differ ($P < 0.05$).

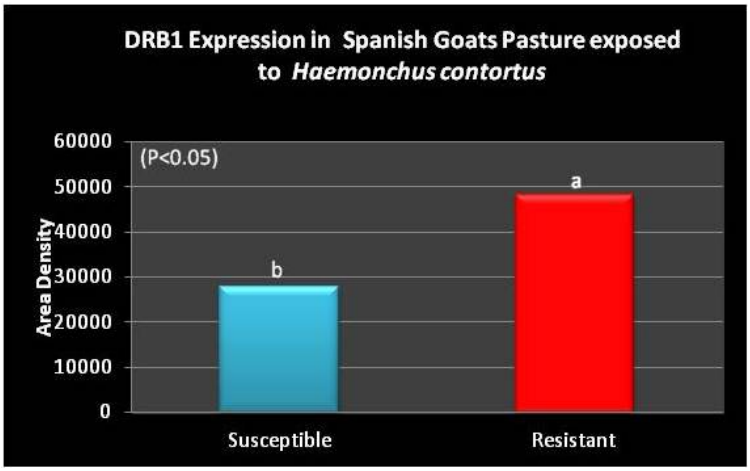

Figure 9. DRB1*1101 Expression in Spanish Goats Pasture exposed to *Haemonchus contortus*

Note. ab, Means with different letters differ (P < 0.05).

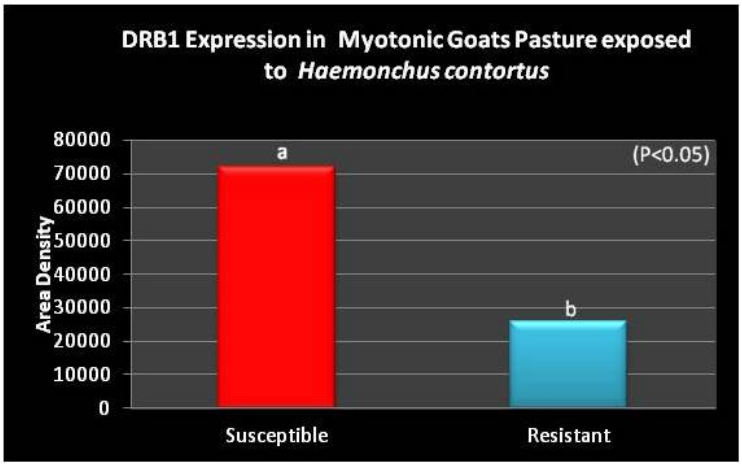

Figure 10. DRB1*1101 Expression in Myotonic Goats Pasture exposed to *Haemonchus contortus*

Note. ab, Means with different letters differ (P < 0.05).

5. Conclusion

The results of this study indicated that the cross species oligonucleotide primers designed from the conserved regions of the DRB1*1101 genes can successfully be used to isolate the goat DRB1*1101, from blood samples, and used as a biomarker to evaluate gene expression in response to *Haemonchus contortus* infection in meat goats. Based on our findings, the DRB1*1101 allele could potentially be used as a genetic marker for susceptibility or resistance to *Haemonchus contortus* between and within breeds. *Haemonchus contortus* continues to be a problem for the small ruminant industry. As GIN resistance to anthelmintics prevails, cost to the industry does. Persistent evaluation of the DBR1 locus will help us to better understand its role in disease resistance or susceptibility of goats to *Haemonchus contortus*.

References

Altschul, S. F., Gish, W., Miller, W., Myers, E. W., & Lipman, D. J. (1990). Basic local alignment search tool. *J Mol Biol, 215*(3), 403-410. http://dx.doi.org/S0022-2836(05)80360-2

Andersson, L., & Rask, L. (1988). Characterization of the MHC class II region in cattle. The number of DQ genes varies between haplotypes. *Immunogenetics, 27*(2), 110-120. http://dx.doi.org/10.1007/BF00351084

Axtner, J., & Sommer, S. (2012). The functional importance of sequence versus expression variability of MHC alleles in parasite resistance. *Genetica, 140*(10-12), 407-420. http://dx.doi.org/10.1007/s10709-012-9689-y

Brown, J. H., Jardetzky, T. S., Gorga, J. C., Stern, L. J., Urban, R. G., Strominger, J. L., & Wiley, D. C. (1993).

Three-dimensional structure of the human class II histocompatibility antigen HLA-DR1. *Nature, 364*(6432), 33-39. http://dx.doi.org/10.1038/364033a0

Brown, J. H., Jardetzky, T. S., Gorga, J. C., Stern, L. J., Urban, R. G., Strominger, J. L., & Wiley, D. C. (2015). Pillars article: three-dimensional structure of the human class II histocompatibility antigen HLA-DR1. Nature. 1993. 364: 33-39. *J Immunol, 194*(1), 5-11.

Buítkamp, J., Filmether, P., Stear, M. J., & Epplen, J. T. (1996). Class I and class II major histocompatibility complex alleles are associated with faecal egg counts following natural, predominantly Ostertagia circumcincta infection. *Parasitol Res, 82*(8), 693-696. http://dx.doi.org/10.1007/s004360050187

Clark, C. H., Keisel, G. K., & Goby, C. H. (1962). Measurements of blood loss caused by *Haemonchus contortus* infection in sheep. *Am J Vet Res, 23*, 977-980.

Corley, M., & Jarmon, A. (2012a). A Common Beta Tubulin Isotype-1 Gene Single Nucleotide Polymorphism as a Tool for Detection and Quantitation of Anthelmintic Resistant *Haemonchus contortus* in Grazing Goats. *Journal of Agricultural Science, 4*(6), 1-11. http://dx.doi.org/10.5539/jas.v4n6p1

Corley, M., & Jarmon, A. (2012b). Interleukin 13 as a Biomarker for Parasite Resistance in Goats Naturally Exposed to *Haemonchus contortus*. *Journal of Agricultural Science, 4*(7), 31-40. http://dx.doi.org/10.5539/jas.v4n7p31

Dukkipati, V. S., Blair, H. T., Garrick, D. J., & Murray, A. (2006). 'Ovar-Mhc' - ovine major histocompatibility complex: structure and gene polymorphisms. *Genet Mol Res, 5*(4), 581-608.

Hassan, M., Good, B., Hanrahan, J. P., Campion, D., Sayers, G., Mulcahy, G., & Sweeney, T. (2011). The dynamic influence of the DRB1*1101 allele on the resistance of sheep to experimental Teladorsagia circumcincta infection. *Vet Res, 42*, 46. http://dx.doi.org/10.1186/1297-9716-42-46

Keane, O. M., Zadissa, A., Wilson, T., Hyndman, D. L., Greer, G. J., Baird, D. B., ... McEwan, J. C. (2006). Gene expression profiling of naïve sheep genetically resistant and susceptible to gastrointestinal nematodes. *BMC Genomics, 7*, 42. http://dx.doi.org/10.1186/1471-2164-7-42

Le Jambre, L. F. (1995). Relationship of blood loss to worm numbers, biomass and egg production in Haemonchus infected sheep. *Int J Parasitol, 25*(3), 269-273. http://dx.doi.org/0020751994001188

Li, R. Y., Hui, W. Q., Jia, B., Shi, G. Q., Zhao, Z. S., Shen, H., ... Li, H. T. (2011). The relationship between MHC-DRB1 gene second exon polymorphism and hydatidosis resistance of Chinese Merino (Sinkiang Junken type), Kazakh and Duolang sheep. *Parasite, 18*(2), 163-169. http://dx.doi.org/10.1051/parasite/2011182163

Outteridge, P. M., Andersson, L., Douch, P. G., Green, R. S., Gwakisa, P. S., Hohenhaus, M. A., & Mikko, S. (1996). The PCR typing of MHC-DRB genes in the sheep using primers for an intronic microsatellite: Application to nematode parasite resistance. *Immunol Cell Biol, 74*(4), 330-336. http://dx.doi.org/10.1038/icb.1996.59

Paterson, S., Wilson, K., & Pemberton, J. M. (1998). Major histocompatibility complex variation associated with juvenile survival and parasite resistance in a large unmanaged ungulate population. *Proc Natl Acad Sci, USA, 95*(7), 3714-3719. http://dx.doi.org/10.1073/pnas.95.7.3714

Polat, M., Aida, Y., Takeshima, S. N., Aniwashi, J., & Halik, M. (2015). The diversity of major histocompatibility complex class II DRB1 gene in sheep breeds from Xinjiang, China. *Tissue Antigens, 85*(1), 50-57. http://dx.doi.org/10.1111/tan.12480

Rowe, J. B., Nolan, J. V., de Chaneet, G., Teleni, E., & Holmes, P. H. (1988). The effect of haemonchosis and blood loss into the abomasum on digestion in sheep. *Br J Nutr, 59*(1), 125-139. http://dx.doi.org/S0007114588000170

Saddiqi, H. A., Jabbar, A., Sarwar, M., Iqbal, Z., Muhammad, G., Nisa, M., & Shahzad, A. (2011). Small ruminant resistance against gastrointestinal nematodes: A case of *Haemonchus contortus*. *Parasitol Res, 109*(6), 1483-1500. http://dx.doi.org/10.1007/s00436-011-2576-0

Saddiqi, H. A., Sarwar, M., Iqbal, Z., Nisa, M., & Shahzad, M. A. (2012). Markers/parameters for the evaluation of natural resistance status of small ruminants against gastrointestinal nematodes. *Animal, 6*(6), 994-1004. http://dx.doi.org/10.1017/S1751731111002357

Sayers, G., Good, B., Hanrahan, J. P., Ryan, M., Angles, J. M., & Sweeney, T. (2005). Major histocompatibility complex DRB1 gene: Its role in nematode resistance in Suffolk and Texel sheep breeds. *Parasitology,*

131(Pt 3), 403-409. http://dx.doi.org/10.1017/S0031182005007778

Schwaiger, F. W., Gostomski, D., Stear, M. J., Duncan, J. L., McKellar, Q. A., Epplen, J. T., & Buitkamp, J. (1995). An ovine major histocompatibility complex DRB1 allele is associated with low faecal egg counts following natural, predominantly Ostertagia circumcincta infection. *Int J Parasitol, 25*(7), 815-822. http://dx.doi.org/10.1016/0020-7519(94)00216-B

Stear, M. J., Bairden, K., Bishop, S. C., Buitkamp, J., Epplen, J. T., Gostomski, D., ... Wallace, D. S. (1996). An ovine lymphocyte antigen is associated with reduced faecal egg counts in four-month-old lambs following natural, predominantly Ostertagia circumcincta infection. *Int J Parasitol, 26*(4), 423-428. http://dx.doi.org/10.1016/0020-7519(96)00002-1

Stear, M. J., Belch, A., Donskow-Schmelter, K., Fitton, L. A., Innocent, G. T., Ishikane, C., ... Sayers, G. (2007). Detection of genes with moderate effects on disease resistance using ovine mhc and resistance to nematodes as an example. *Vet Immunol Immunopathol, 120*(1-2), 3-9. http://dx.doi.org/10.1016/j.vetimm.2007.07.012

Stear, M. J., Innocent, G. T., & Buitkamp, J. (2005). The evolution and maintenance of polymorphism in the major histocompatibility complex. *Vet Immunol Immunopathol, 108*(1-2), 53-57. http://dx.doi.org/10.1016/j.vetimm.2005.07.005

Untalan, P. M., Pruett, J. H., & Steelman, C. D. (2007). Association of the bovine leukocyte antigen major histocompatibility complex class II DRB3*4401 allele with host resistance to the Lone Star tick, Amblyomma americanum. *Vet Parasitol, 145*(1-2), 190-195. http://dx.doi.org/10.1016/j.vetpar.2006.12.003

Valilou, R. H., Rafat, S. A., Notter, D. R., Shojda, D., Moghaddam, G., & Nematollahi, A. (2015). Fecal egg counts for gastrointestinal nematodes are associated with a polymorphism in the MHC-DRB1 gene in the Iranian Ghezel sheep breed. *Front Genet, 6*, 105. http://dx.doi.org/10.3389/fgene.2015.00105