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Association between the *Ovine MHC-DRB1* Gene and its Resistance to Gastrointestinal Parasites in Deccani Sheep Raised in Hot Semi-arid Ecosystem of India

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ABSTRACT

Backgroung: Major histocompatibility complex (MHC) is linked with the ability of sheep to resist infection by GIN as measured by feacal egg count. Present study was carried out to genotype Deccani sheep for *DRB1* locus and associate it with parasitic resistance.

Methods: PCR SSCP analysis for *Ovar-DRB1 exon* 2 and part of *intron-1* was carried out under optimum conditions. Effect of genetic and nongenetic factors along with the genotype was performed using linear model of least square analysis by considering log transferred faecal egg count as a dependent variable.

Result: PCR SSCP analysis revealed presence of 14 SSCP patterns. The factors such as age, sex, farm and birth type demonstrated a non significant effect on faecal egg count however, season was observed to be significant source of variation. Further, it was observed that, genotype J and F could be possibly associated with resistance and susceptibility to parasitic infection. Thus, it was inferred that *DRB1* gene is may be associated with immune response to parasitic invasion and selection for genotype associated with *Ovar-Mhc-DRB1* gene could improve the parasitic resistance in Deccani sheep.

Key words: Deccani, Feacal egg count, *Ovar-DRB1* gene, PCR-SSCP.

INTRODUCTION

Sheep husbandry is an important and sustainable livelihood resource for people living in the arid and semi-arid regions of the world since long time. The rearing of small ruminants is very important, since they serve as a lifeline during drought years by providing income and sustenance to the farming community and grazers (Rangnekar, 2006). Sheep population of India is 74.26 million and accounts for almost 13.87 per cent of the total livestock and India ranks second in the world (DAHD, 2019). Deccani sheep is drought resistant sturdy breed of Deccan plateau, particularly Maharashtra state. Gastrointestinal parasites, particularly the gastro intestinal nematodes (GIN) is one of the major constraints in sheep husbandry contributing to overall production losses for the shepherds by affecting the production performance of small ruminants (Kumar *et al.,* 2008). Individual as well as breed wise variations to different parasitic infection resistance and susceptibility are seen in animals (Saddiqi *et al.*, 2011).

Many researchers link the genes in the sheep major histocompatibility complex (MHC*)* with the ability of sheep to resist infection by GIN as measured by feacal egg count (FEC) and revealed polymorphisms in *exon 2* and adjoining *intron 2* of the expressed gene *DRB1*. The *DRB1 exon 2* encodes *β1* domain, which constitutes part of the protein binding receptor (*PBR*) or T-cell receptors of the *DR* molecules and is likely to be related to functionality such as disease resistance/susceptibility (Paterson *et al.*, 1998; Charon *et al.*, 2002; Ashrafi *et al.,* 2014 and Valilou *et al.,* 2015). The *DR* genes are highly polymorphic in nature and proteins encoded by these genes are expressed in elevated concentrations, specifically on the cell membranes of macrophages and *B cells* (Outteridge *et al.*, 1996). The highly variable residues concentrated in this region are in close contact with the peptides presented in the *PBR* or the *TCR* (Brown *et al.*, 1993). In view of this, an investigation was carried out to genotype *exon* 2 of *DRB1* locus of *Ovar-Mhc* and assesses its association with FEC in Deccani sheep.

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MATERIALS AND METHODS

Blood collection, processing and PCR amplification

The present investigation includes total of 50 unrelated Deccani sheep reared at Instructional Livestock Farm

Complex (ILFC), College of Veterinary and Animal Sciences, Udgir and farmer's flock at Village-Valandi in Udgir Tehsil from the period October 2019 to October 2020. About 4-5 ml of blood was collected from all the animals using aseptic conditions from Jugular vein and was then carried to the laboratory on ice, stored at 4°C till DNA isolation was performed. Genomic DNA was isolated using DNA isolation kit (QIAamp DNA Blood Mini Kit) as per manufacturer's instructions. The DNA was quantified using UV spectrophotometer. Sequence specific primers reported by Gowane *et al.* (2017a) and Gowane *et al.* (2017b) were utilized to amplify the region of interest. The polymerase chain reaction (PCR) was carried out in a total volume 50 µl solution containing 1 µl template DNA, 10X buffer with $Mgcl_2$ 5 µl, 2.5 Mm dNTPs (100 µM each) 1.0 µl, forward primer (20 pmol/µl) 1.0 µl, reverse primer (20 pmol/µl) 1.0 µl and *Taq* DNA polymerase 1.0 µl. The denaturation was done at 94°C, anneling at 60°C and extension at 72°C for about 35 cycles. PCR products were separated by electrophoresis on 2.5% agarose gel. Genotyping of samples was carried out at Central Sheep and Wool Research Institute, Avikanagar, Rajasthan.

PCR-Single strand conformation polymorphism (PCR-SSCP)

PCR- Single strand conformation polymorphism (PCR-SSCP) was performed to detect variation in gene. Aliquots of 10 µl purified PCR products obtained after gel extraction were mixed with 10 µl loading dye, heated for 5 min at 95°C and chilled on ice for 5 min. The samples were separated by an electrophoresis on a 12% neutral polyacrylamide gel (Acrylamide:Bis-Acrylamide = 29:1) at 100 volt for 24 hours. The gels were stained with silver staining to identify SSCP patterns. SSCP patterns were determined by visual/manual analysis of bands.

Faecal sample examination

Faecal samples were collected in aseptic tubes and transferred to the refrigerator to be stored at 4°C for further processing. Faecal eggs were counted by Stoll's dilution method (Soulsby, 1982) as per standard protocol. The data on the faecal egg count was recorded and normalised by log transformation as follows:

$$LFEC = \log_e (FEC + 100)$$

Statistical analysis

Linear model of least square analysis (Harvey, 1990) was performed to establish the association between different fixed effects and parasitic resistance indicated by least squares mean of FEC (LFEC). Statistical model considered for the present analysis was;

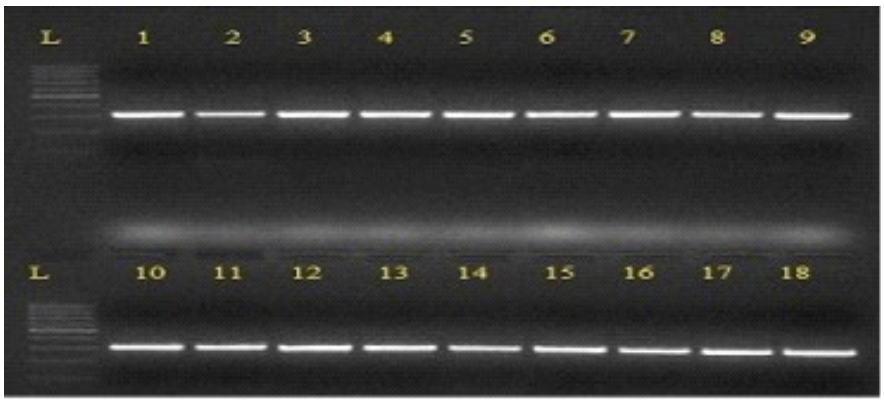
$$\hat{Y} = \mu + A_i + B_j + C_k + D_l + E_m + F_n + e_{ijklmn}$$

Where,
$\hat{Y}$ = LFEC, µ is global mean.
A_i = i^{th} effect of season on LFEC.
B_j = j^{th} effect of sex on LFEC.
C_k = k^{th} effect of age group on LFEC.
D_l = l^{th} effect of farm on LFEC.
E_m = m^{th} effect of birth type on LFEC.
F_n = n^{th} effect of genotype on LFEC.
e_{ijklmn} = Error NID (o,σ^2e).

Results and Discussion

Genotyping of deccani sheep for *Ovar-DRB1* locus

The DNA samples were visualized under UV illuminator and further on gel documentation system. After optimization of PCR thermal cycle conditions a PCR product 301 bp was obtained and confirmed with a 100 bp ladder (Fig 1). On visualization of polyacrylamide gel, fourteen SSCP patterns were obtained and frequency of pattern D was highest while that of pattern L was lowest (Fig 2 and Fig 3). Each pattern displayed variable number of bands in variable arrangements (Table 1). In accordance with the present findings, Bhide and Mikula (2005), studied polymorphism in DRB1 region in Valachian sheep and revealed 25 different SSCP patterns in 400 sheep. Similarly, SSCP patterns ranging from 11 to 31 were reported in different breeds of sheep (Shaobin *et al.*, 2015, Zamani *et al.*, 2016, Gowane *et al.,* 2017a).



(L for Ladder 100 bp; Lane 1 and 2 PCR products).

Fig 1: PCR product of 301 bp for *Ovar-DRB1 exon 2* gene.

Seasonal variations in the faecal egg count (FEC)

The range of FEC observed was zero to 6600 eggs and maximum FEC was observed for a sheep of SSCP variant J in monsoon season. Factor season was found to have a significant source of variation on FEC as compared to other genetic and non-genetic factors. The mean egg count of parasite in monsoon season was significantly higher followed by winter and least in summer (Table 2). Monsoon is the season that is more favorable for the parasites to develop, survive and infect the animals. Presence of different stages of parasites makes them possible to infect animals, leading to presence of higher load of parasitic eggs in faeces, as evident in our findings. The findings are in accordance with Swarnkar and Singh (2014); Molla and Bandyopadhyay (2016) and Dappawar *et al.* (2018) in different breeds of sheep. It was inferred that, young animals demonstrated slightly higher FEC than the lambs and low in adult animals in monsoon season. In summer season, FEC was highest in lambs followed by adults and least in young animals. Adult animals displayed a higher FEC in winter season followed by lambs and young (Table 3). The results are in accordance with McManus *et al.* (2009) and Dappawar *et al.* (2018) were in no significant association was reported between FEC and age of animals.

It was also observed that males displayed a high count of parasitic eggs than females throughout all seasons however; it was statistically non-significant (Table 4). Different hormonal status in sexes affects the immunological responses of sheep to nematodes (Gauly *et al.*, 2006). Thus, it is common to see the males being more prone to parasitic invasion than females. It has also been reported that difference between females and males in parasite susceptibility are probably caused by a difference in

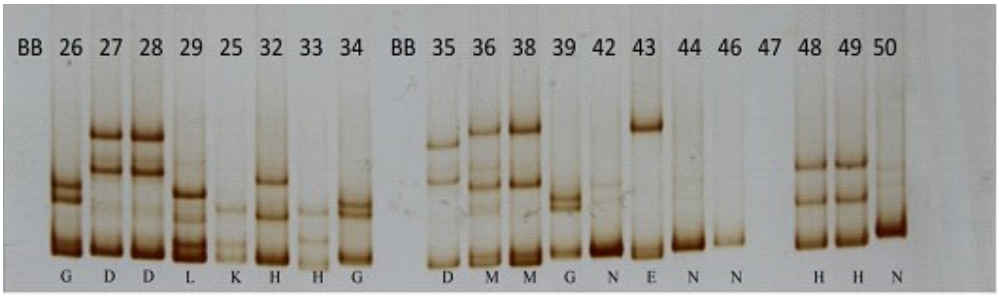
Table 1: Details of band pattern for PCR-SSCP.

Pattern	Numbers of bands	Number of observation	Frequency (%)
A	4	2	4
B	4	2	4
C	3	3	6
D	3	9	18
E	3	2	4
F	3	3	6
G	3	7	14
H	3	8	16
I	3	2	4
J	4	2	4
K	3	2	4
L	3	1	2
M	3	3	6
N	3	4	8

Table 2: Mean values of FEC and LFEC in different season.

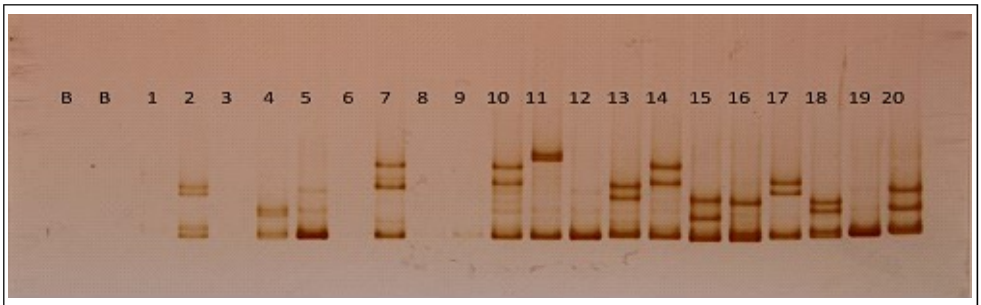
Season	FEC	LFEC**
Monsoon	916±157.385	6.54±0.130[a]
Winter	96±11.051	5.25±0.139[b]
Summer	76±10.902	5.14±0.139[b]

(a, b Means with different superscripts in a column differ significantly **P<0.01).



(Lane 1 Sample number, BB- Blank; Lane 2 SSCP patterns assigned to variants).

Fig 2: SSCP patterns of PCR product for *Ovar-DRB1 exon 2* gene.



(Lane 1 Sample number, B-blank).

Fig 3: SSCP patterns of PCR product for *Ovar-DRB1 exon 2* gene.

behaviour, morphology and physiological status of sex (Zuk and McKean 1996). Similar results were reported by McManus *et al.* (2009) and Dappawar *et al.* (2018).

Further, the animals born as singles/giving birth to singles observed lower FEC during all seasons as compared to the animals born as multiples/giving birth to multiples (Table 5). Romjali *et al.* (1997) and Haile *et al.* (2007) explained that the differences in FEC may be contributed to better rearing and nutrition conditions in the singletons in comparison to the multiples. Similar results were reported in Brazilian sheep (McManus *et al.*, 2009). Frequency of fourteen SSCP patterns across different seasons revealed that, pattern G, I and M had higher FEC in monsoon (Table 6). These results are similar to those reported by Sayers *et al.* (2005) in Texel sheep and Kumari *et al.* (2020) in Munjal sheep.

Association of FEC with genotype

FEC ranged from zero to 6600 in the study leading to huge variation in the data. This led to a high standard error associated with FEC. In order to normalize the data, a conversion of FEC to LFEC was done. Linear model of least square analysis was performed to establish the association between genotype of *exon 2 Ovar-DRB1* gene

Table 3: Mean values of FEC and LFEC for different age groups.

Age group	Monsoon		Winter		Summer	
	FEC	LFEC	FEC	LFEC	FEC	LFEC
Lamb (0 to 6 months) n= 13	1130.77±262.24	6.752±0.281	84.615±15.38	5.170±0.094	84.62±22.21	5.125±0.127
Young (6 months to 1 yr) n= 12	1133.33±250.25	6.777±0.288	58.333±24.10	5.111±0.137	83.333±19.30	4.986±0.119
Adult (above 1 yr) n= 25	700±256.12	6.179±0.187	108.00±17.24	5.239±0.095	80.00±16.33	5.091±0.093

(n= no. of animals in each group).

Table 4: Mean values of FEC and LFEC for sex group.

Sex	Monsoon		Winter		Summer	
	FEC	LFEC	FEC	LFEC	FEC	LFEC
Male n= 5	960±380.263	6.485±0.577	140±40	5.403±0.214	80±37.416	5.102±0.215
Female n= 45	911.111±170.834	6.470±0.146	91.111±11.380	5.166±0.064	75.556±11.527	5.071±0.066

(n= no. of animals in each group).

Table 5: Mean values of FEC and LFEC for Birth type.

Birth type	Monsoon		Winter		Summer	
	FEC	LFEC	FEC	LFEC	FEC	LFEC
Single n= 47	914.634±186.415	6.442±0.157	90.243±11.998	5.160±0.068	68.292±11.282	5.036±0.067
Multiple n= 3	950±287.228	6.729±0.137	133.333±33.333	5.385±0.175	150±34.156	5.453±0.182

(n= no. of animals in each group).

Table 6: Mean values of FEC and LFEC for SSCP patterns.

Patterns	Monsoon		Winter		Summer	
	FEC	LFEC	FEC	LFEC	FEC	LFEC
A	50±50.001	4.952±0.347	50±50	4.951±0.346	150±50.001	5.501±0.202
B	850±550	6.652±0.661	50±50	4.951±0.346	150±50.001	5.501±0.202
C	600±251.661	6.432±0.335	100±57.735	5.202±0.320	33.333±33.335	4.836±0.231
D	800±192.930	6.602±0.231	77.778±27.778	5.080±0.159	66.667±23.570	5.035±0.142
E	750±150	6.729±0.178	50±50	4.951±0.346	100±100	5.154±0.549
F	500±450.924	5.739±0.812	66.667±33.334	5.067±0.231	100±57.735	5.202±0.320
G	1128.571±434.639	6.694±0.420	100±37.796	5.175±0.208	71.428±28.571	5.059±0.169
H	700±225.198	6.407±0.286	112.5±22.658	5.313±0.120	50±26.726	4.915±0.157
I	1150±850	6.821±0.829	150±50	5.501±0.202	100±100	5.154±0.549
J	4000±2600	8.062±0.748	150±50	5.501±0.202	150±50.001	5.501±0.202
K	300±100	5.959±0.255	50±50	4.951±0.346	50±50.001	4.951±0.346
L	0±0	4.605±0	200±0	5.703±0	200±0	5.703±0
M	1200±850.490	6.738±0.638	166.667±33.334	5.568±0.135	0±0	4.605±0
N	1000±348.807	6.595±0.667	75±47.871	5.053±0.271	75±25	5.125±0.173

Table 7: LSM values of genotypes SSCP pattern.

SSCP pattern	LS means & SE for FEC**
A	5.296±0.312
B	5.864±0.312
C	5.460±0.262
D	5.601±0.162
E	5.520±0.319
F	5.255±0.255
G	5.804±0.177
H	5.696±0.173
I	5.598±0.339
J	6.516±0.309
K	5.449±0.312
L	5.501±0.416
M	5.824±0.293
N	5.658±0.226

(LFEC= Season*sex*age*farm*birth type*genotype; ** Highly significnat).

and parasitic resistance indicated by LFEC. The coefficient of determination (R^2 value) for model considered in the study was 54% (P<0.01). Least square means of LFEC for genotype J was highest (6.516± 0.309) followed by genotype B (5.864±0.312) and genotype F (5.255±0.255). It was inferred from the study that the genotype J may be associated with higher LFEC (P<0.01) while genotype F may be associated with reduced LFEC. Therefore, the animals with genotype F for *exon 2 Ovar-DRB1* could be said to be resistant to parasitic invasion as attributed to *Ovar-Mhc* (Table 7).

Conclusion

Molecular characterization of *Ovar-DRB1 exon 2* revealed fourteen genotypes patterns represented by PCR-SSCP. The study suggested possible association of genotype 'F' and genotype 'J' with parasitic resistance and susceptibility in Deccani sheep. Findings revealed that *DRB1* gene is associated with immune response to parasitic invasion and selection for genotype associated with *Ovar-Mhc-DRB1* gene could improve the parasitic resistance in Deccani sheep. However, parasitic resistance is a lowly heritable trait affected by many environmental and non genetic factors therefore large scale study on a considerable number of samples is needed for better confirmation.

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Conflict of interest

All authors declare that they have no conflicts of interest.

References

Ashrafi, F., Hashemi, A., Mardani, K. and Darvishzadeh, R. (2014). Study on genetic variability in *Mhc-DRB1 second exon* in makuie sheep breed population. Genetica. 46: 269-275. doi: 10.2298/GENSR1401269A.

Bhide, M.R. and Mikula, I. (2005): Asymmetric PCR-SSCP: a Useful Tool for Detection of *OLA-DRB1* (Mhc class II) Gene Polymorphism in Slovak Improved Valachian Sheep. Acta Veterinaria Brno. 74: 275-278.

Brown, J.H., Jardetzky, T.S., Gorga, J.C. and Stern, L.J. (1993). Three-dimensional structure of the human class II histocompatibility antigen HLA-DR1. Nature. 364: 33-39.

Charon, K.M., Moskwa, B., Rutkowski, R., Gruszczynskal, J. and Swiderek, W. (2002). Microsatellite polymorphism in DRB1 gene (*Mhc* class II) and its relation to nematode faecal egg count in Polish Heath Sheep. Journal of Animal and Feed Science. 11: 47-58.

Dappawar, M.K., Khillare, B.S., Narladkar, B.W. and Bhangale, G.N. (2018). Prevalence of gastrointestinal parasites in small ruminants in Udgir area of Marathwada. Journal of Entomology and Zoology Studies. 6(4): 672-676.

Gauly, M., Schackert, M., Hoffmann, B. and Erhardt, G. (2006). Influence of sex on the resistance of sheep lambs to an experimental Haemonchus contortus infection. Deut Tierarztl Woch. 113: 178-181.

Gowane, G.R., Najif Akram, L.L., Prince, L., Prakash, V. and Kumar, A. (2017a). *Ovar-Mhc* Polymorphism in Malpura and Avikalin Sheep vaccinated for Peste des Petits Ruminants (PPR) Virus. Animal Biotechnology. 28(4): 306-314.

Gowane, G.R., Najif Akram, L.L., Prince, L., Ved Prakash and Arun Kumar. (2017b). Genetic appraisal of serological response post vaccination against enterotoxaemia (ET) in Malpura and Avikalin sheep. Tropical Animal Health Production. 49: 867-873.

Haile, A., Tibbo, M., Baker, R.L. and Rege, J.E.O. (2007). Effects of nongenetic factors on responses to gastro-intestinal nematode infections in Ethiopian sheep. Tropical Animal Health Production. 39(6): 411-417.

Harvey, W.R. (1990). User's Guide for LSMLMW, MIXMDL, PC-2I Version. Columbus.

Kumar, R.R., Yadav, C.L., Garg, R., Banerjee, P.S. and Vatsya, S. (2008). Prevalence of gastro-intestinal nematodes in sheep and goat in some parts of north-west India. Indian Journal of Animal Science. 78(11): 1244-1246.

Kumari, A., Kumar, A., Tomar, P., Baro, D., Grewal, S. and Sangwan, M.L. (2020). *Mhc-DRB1 exon 2* polymorphism and its association with faecal egg count of *Haemonchus contortus* in Munjal sheep. Indian Journal of Experimental Biology. 58: 365-369.

McManus, C., Helder, L., Samuel, R.P., Amaury, A.D.O., Hymerson, C.A. and Cristiano, B.D.M. (2009). Genetic factors of sheep affecting gastrointestinal parasite infections in the Distrito Federal, Brazil. Veterinary Parasitology. 166: 308-313.

Molla, S.H. and Bandyopadhyay, P.K. (2016). Prevalence of gastro-intestinal parasites in economically important Bonpala sheep in India. IOSR-Journal of Agriculture and Veterinary Science. 9(1): 87-93.

Outteridge, P.M. andersson, L., Douch, P.G.C. and Green, R.S. (1996). The PCR typing of *Mhc-DRB* genes in the sheep using primers for an *intronic* microsatellite: Application to nematode parasite resistance. Immunology and Cell Biology. 74: 330-336.

Paterson, S., Wilson, K. and Pemberton, J.M. (1998). Major histocompatibility complex variation associted with juvenile survival and prasite resistance in a large ungulate population (*Ovis aries* L.). Proceedings of the National Academy of Sciences. U.S.A. 95: 3714-3719. doi: 10.1073/pnas.95.7.3714.

Rangnekar, D.V. (2006). Livestock in the livelihoods of the underprivileged communities in India: A review. ILRI (aka ILCA and ILRAD).

Romjali, E., Pandey, V.S., Gatenby, R.M., Doloksaribu, M., Sakul, H., Wilson, A. and Verhulst, A. (1997). Genetic resistance of different genotypes of sheep to natural infections with gastro-intestinal nematodes. Animal Science. 64: 97-104

Saddiqi, H.A., Abdul, J., Muhammad, S., Zafar I., Ghulam, M., Mahrun, N. and Aasif, S. (2011). Small ruminant resistance against gastrointestinal nematodes: A case of *Haemonchus contortus*. Parasitology Research. 109: 1483-1500.

Shaobin, L., Yuzhu, L., Jiang, H., Jiqing, W., Xiu, L. and Wei, Y. (2015). Variation of *DRB1* Gene in Tibetan Sheep. Journal of Life Sciences. 9: 255-261.

Soulsby, E.J.L. (1982). Helminths, Arthropods and Protozoa of Domesticated Animals. 7th edition ELBS and Bailliere Tindall, London. pp. 765-768.

Swarnkar, C.P. and Singh, D. (2014). Influence of annual rainfall on epidemiology of gastrointestinal parasites in sheep flocks of Rajasthan. Indian Journal of Animal Sciences. 84(11): 1171-1176.

Sayers, G., Good, B., Hanrahan, J.P., Ryan, M., Angles, J.M. and Sweeney, T. (2005). Major histocompatibility complex DRB1 gene: Its role in nematode resistance in Suffolk and Texel sheep breeds. Prasitology. 131: 403-409.

Valilou, R.H., Rafat, S.A., Notter, D.R., Shojda, D., Moghaddam, G. and 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. Frontiers Genetics. 6: 105.

Zamani, P., Hosein S. D., Abbas D. and Alireza A. (2016). Ovine *DRB1* polymorphism and its associations with body weight, milk contents and immunological parameters. Annuals of Animal Science. 16(2): 425-438.

Zuk, M. and McKean, K.A. (1996). Sex differences in parasite infections: Patterns and processes. International Journal of Parasitology. 26: 1009-1023.