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Analysis of the Relationship between MHC-DRB1 Gene Polymorphism and Hydatidosis in Kazakh Sheep

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ABSTRACT : The objective of this work was to analyze the relationship between ovine major histocompatibility complex (MHC) *DRB*1 gene polymorphism and genetic resistance to hydatidosis in Kazakh sheep. The Ovar (ovine MHC) class II *DRB*1 second exon was amplified by polymerase chain reaction (PCR) from DNA samples of 702 Kazakh sheep, including 302 sheep with hydatidosis and 400 health controls. PCR products were characterized by the restriction fragment length polymorphism (RFLP) technique using five restriction enzymes, i.e., *Mva*I, *Hae*III, *Sac*I, *Sac*II and *Hin*1I, yielding 14 alleles and 28 genotypes. Comparing the frequency of genotypes in hydatidosis sheep with the control group, it was found that the genotype frequencies of *Mva*Ibc, *Hin*1Iab, *Sac*IIab, *Hae*IIIde, *Hae*IIIdf and *Hae*IIIdd in control sheep were significantly ($p<0.01$) higher than in hydatidosis sheep, indicating that a significant correlation existed between these genotypes and resistance to hydatidosis. Genotype frequencies of *Mva*Ibb, *Sac*IIaa, *Hin*1Ibb and *Hae*IIIef in sheep with hydatidosis were extremely significantly ($p<0.01$) higher than in the control group, and the genotype frequency of *Hae*IIIab was significantly higher ($p<0.05$), indicating that a marked correlation existed between these genotypes and susceptibility to hydatidosis. By way of analyzing haplotype with these resistant genotypes, the hydatidosis resistant haplotype *Mva*Ibc-*Sac*IIab-*Hin*1Iab of Kazakh sheep was screened out, and then verified through artificial hydatid infection in sheep. The results indicated that the infection rate of sheep with the resistant haplotype of hydatidosis was significantly lower ($p<0.01$) than without this resistant haplotype. It showed that the genic haplotype *Mva*Ibc-*Sac*IIab-*Hin*1Iab of *Ovar-DRB*1 exon 2 was the resistant haplotype of hydatidosis in Kazakh sheep. (**Key Words :** Major Histocompatibility Complex, Ovar (Ovine MHC), Restriction Fragment Length Polymorphism)

INTRODUCTION

Hydatidosis (*Echinococcus granulosus*) is recognized as one of the world's major zoonoses, and is distributed all over the world (Rausch 1995; Andersen et al., 1997; Dalimi et al., 2002; Eckert and Deplazes 2004; Jenkins et al., 2005). Sinkiang Autonomous Region of China is one of the prevalent areas of hydatidosis. In sheep, the overall prevalence rate was 38.89-61.25% (Li et al., 2005) for hydatid cysts. Kazakh sheep are a native species of sheep of Sinkiang, growing in closed conditions, the genetic background is relatively simple and this species can greatly utilize roughage but meanwhile has strong disease resistance and good performance (Adeli et al., 2009). The high prevalence of hydatidosis seriously hinders the success of sheep breeding , and also affects the quality of life of herdsmen.

In recent years, research on the MHC as candidate genes of disease resistance and susceptibility has become a major focus in animal breeding. The MHC is a multigene family controlling immunological self/non-self recognition. Amongst them are the genes that encode the cell surface glycoproteins that present peptides of foreign and self proteins to T cells, thereby controlling all specific immune responses, both cell- and antibody-mediated (Klein, 1986). A striking characteristic of MHC genes is their extreme polymorphism. Exon 2 of the *Ovar-DRB*1 gene codes for part of the MHC class II antigen binding cleft and over 80 alleles have been identified at this locus in sheep (Sayers et al., 2005). Diversity driven by pathogens implies a strong association between MHC alleles and patterns of resistance to specific autoimmune or infectious diseases. Such a link was first shown for chickens in which the B21 haplotype

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(MHC class IIB) confers the strongest resistance to the herpes virus responsible for Marek's disease (Briles et al., 1977; Longenecker and Gallatin, 1978). Equally famous is the role of class I chicken MHC in providing resistance to the Rous sarcoma virus (Schierman and Collins, 1987; Kaufman and Venugopal, 1998). The polymorphism of the *Ovar-DRB*1 gene plays an important role in resistance to nematode infection in the Suffolk breed (Sayers et al., 2005).

In this study, the objective was to analyse the relationship between ovine MHC-*DRB*1 gene polymorphism and genetic resistance to hydatidosis in Kazakh sheep, and to screen out and verify the haplotype of *Mva*Ibc-*Sac*IIab-*Hin*1Iab which is resistant to hydatidosis. This work could play an important role in breeding a new kind of sheep which could resist hydatidosis.

MATERIALS AND METHODS

Animal sampling and sample preparation

The blood samples were collected from 702 one-year-old Kazakh sheep donated from the regimental farm 74 of the Agricultural Construction Division 4 in Sinkiang. The sheep with hydatidosis and healthy sheep were distinguished by an ovine hydatidosis ELISA kit (Shenzhen Combined Biotech Co., Ltd.). Of all the selected sheep, there were 302 hydatidosis sheep and 400 healthy controls. Samples of genomic DNA were obtained from whole blood, using the preparation procedure of Liu et al. (1997), and stored in a -20°C freezer until analysis. The major materials and reagents were obtained from Shanghai Sangon Biological Engineering Technology and Service Co., Ltd..

Design of *Ovar-DRB*1 exon 2 specific primers and PCR amplification

The second exon of *Ovar-DRB*1 was amplified by the PCR technique in two stages. The first round of PCR was performed with primers *OLA-ERB*1 (GC) 5′-CCG GAA TTC CCG TCT CTG CAG CAC ATTTCT T-3′ and HL031 5′-TTT AAA TTC GCG CTC ACCTCG CCG CT-3′ (adopted from Konnai et al., 2003). We subjected 100 ng of genomic DNA to amplification by PCR in a total volume of 20 μl, including 1.5 m*M* $MgCl_2$, and 120 μ*M* dNTP, to which 0.2 m*M* of each primer and 1.5 U of Taq polymerase had been added. Reactions were performed in a thermocycler under the following conditions: one cycle of incubation for 5 min at 94°C, followed by 15 cycles of 94°C for 30 s, 50°C for 30 s, and 72°C for 60 s, with final extension at 72°C for 10 min. We used 3 μl of the resulting mixture, plus primers *OLA-ERB*1 (GC) and *OLA-XRB*I (5′-AGC TCG AGC GCT GCA CAG TGAAAC TC-3′) (adopted from Konnai et al., 2003) for the second round of PCR. The conditions for the second round of PCR were one cycle for 5 min at 94°C, followed by 30 cycles of 94°C for 30 s, 63°C for 30 s, and 72°C for 60 s with final extension at 72°C for 10 min.

Polymorphism detection by RFLP

Ten microliters of the mixture after the second round of PCR were digested for 4 h at 37°C with 5 U of *Mva*I, *Hae*III, *Sac*I, *Sac*II and *Hin*1I respectively in a total volume of 20 μl. Samples were resolved by a 2.5 or 3% agarose gel electrophoresis.

Statistical analysis

The hydatidosis infection rate of sheep was checked by Fisher's exact test after the artificial hydatid infection experiment. The other data were analyzed by Chi-Square test, $p<0.05$ was considered to be statistically significant and $p<0.01$ was considered as extremely significant.

RESULTS

The result of PCR amplification

*Ovar-DRB*1 exon 2 was amplified by PCR with the primer of *OLA-ERB*1, *OLA-HL*031 and *OLA-XRB*I; one specific band of 296 bp was observed by 1.5% agarose gel electrophoresis (Figure 1).

The result of PCR-RFLP

When each amplified product was cleaved by the restriction enzymes, the cleavage map and allele denomination ways were in accordance with Konnai et al. (2003). The genotypes of restriction enzyme *Sac*I, *Hin*1I, *Mva*I, *Sac*II (Table 1) and *Hae*III (Table 2) were observed, yielding 2, 2, 2, 2 and 6 alleles and 3, 3, 3, 3 and 16

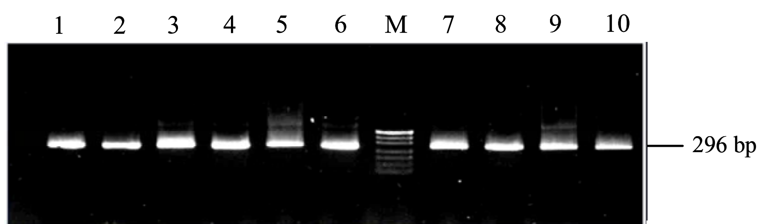

Figure 1. Electrophoretic patterns of PCR product of the second exon of *Ovar-DRB*1 in Kazakh sheep, M: PUC19 DNA marker.

Table 1. The genotypes of PCR-RFLP in the second exon of the *Ovar-DRB*1 gene

Restriction enzymes	The genotypes of each restriction enzyme		
*Sac*I	aa (296 bp)	ab (296 bp/208 bp/88 bp)	bb (208 bp/88 bp)
*Hin*1I	aa (296 bp)	ab (296 bp/178 bp/118 bp)	bb (178 bp/118 bp)
*Mva*I	bb (123 bp/87 bp/86 bp)	bb (210 bp/123 bp/87 bp/86 bp)	cc (210 bp/86 bp)
*Sac*II	aa (296 bp)	ab (296 bp/229 bp/69 bp)	bb (229 bp/69 bp)

Table 2. The genotypes of PCR-RFLP by restriction enzyme *Hae*III in the second exon of the *Ovar-DRB*1 gene

Genotypes	Restriction fragments	Genotypes	Restriction fragments
*Hae*III aa	173 bp/71 bp/48 bp/4 bp	*Hae*III ae	173 bp/159 bp/71 bp/66 bp/48 bp/4 bp
*Hae*III cc	159 bp/137 bp	*Hae*III af	173 bp/159 bp/71 bp/52 bp/48 bp/14 bp/4 bp
*Hae*III dd	159 bp/123 bp/14 bp	*Hae*IIIbc	173 bp/159 bp/137 bp/123 bp
*Hae*III ee	159 bp/71 bp/66 bp	*Hae*III ce	159 bp/137 bp/71 bp/66 bp
*Hae*III ff	159 bp/71 bp/52 bp/14 bp	*Hae*III cf	159 bp/137 bp/71 bp/52 bp/14 bp
*Hae*III ab	173 bp/123 bp/71 bp/48 bp/4 bp	*Hae*III de	159 bp/123 bp/71 bp/66 bp/14 bp
*Hae*III ac	173 bp/159 bp/137 bp/71 bp/48 bp/4 bp	*Hae*III df	159 bp/123 bp/71 bp/52 bp/14 bp
*Hae*III ad	173 bp/159 bp/123 bp/71 bp/48 bp/4 bp	*Hae*III ef	159 bp/71 bp/66 bp/52 bp/14 bp

genotypes, respectively, and their genotypic restriction map is shown in Figure 2, 3, 4, 5 and 6 respectively.

Cloning and sequencing

The predicted RFLP profiles of *Ovar-DRB*1 alleles were verified by sequencing the cloned products amplified, and all the observed patterns of fragments matched exactly with those predicted from DNA sequences.

The result of Chi-Square fitness test

By Chi-Square fitness test, *ovar-DRB*1 exon 2 of Kazakh sheep was analyzed to determine whether it was in Hardy-Weinberg balance. The Chi-Square values of the five restriction enzymes *Mva*I, *Sac*I, *Sac*II, *Hin*1I and *Hae*III were 173.85 ($p<0.01$), 9.24 ($p<0.01$), 0.33 ($p>0.05$), 5.84 ($p>0.05$) and 633.20 ($p<0.01$), respectively. The results suggested that the sites of restriction enzymes *Sac*II and *Hin*1I were within Hardy-Weinberg balance, while the sites

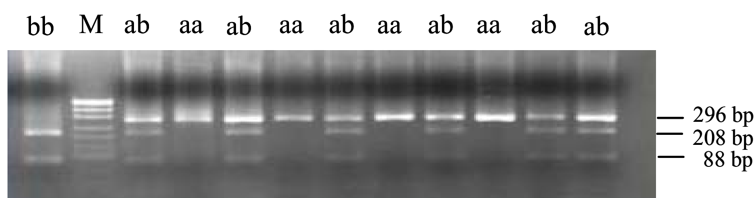

Figure 2. Electrophoretic patterns of the second exon of *Ovar-DRB*1 digested with *Sac*I in Kazakh sheep, M: puc19 DNA marker.

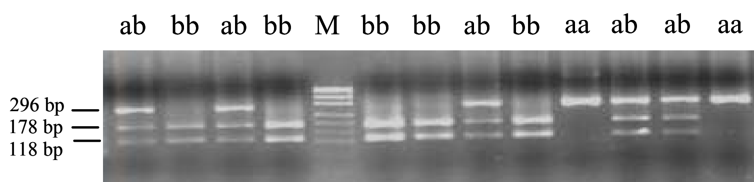

Figure 3. Electrophoretic patterns of the second exon of *Ovar-DRB*1digested with *Hin*1I in Kazakh sheep, M: puc19 DNA marker.

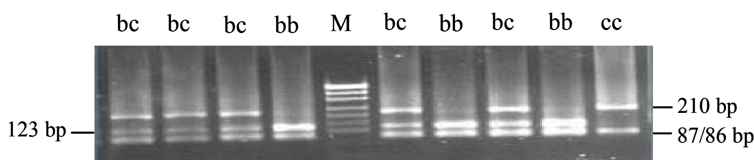

Figure 4. Electrophoretic patterns of the second exon of *Ovar-DRB* digested with *Mva*I in Kazakh sheep, M: puc 19DNA marker.

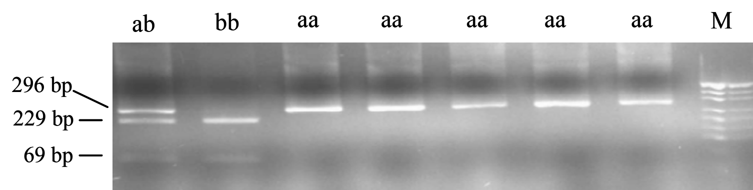

Figure 5. Electrophoretic patterns of the second exon of *Ovar-DRB*1 digested with *Sac*II in Kazakh sheep, M: puc19 DNA marker.

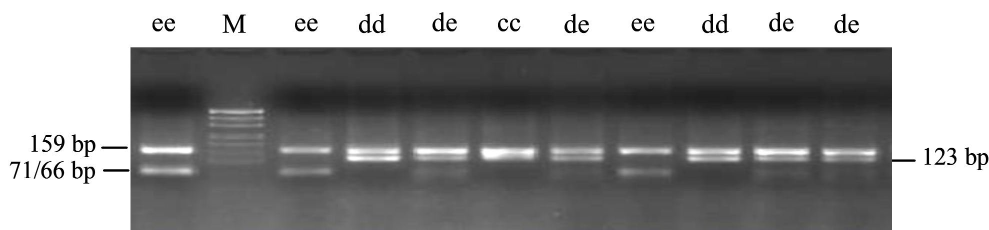

Figure 6. Electrophoretic patterns of the second exon of *Ovar-DRB*1 digested with *Hae*III in Kazakh sheep, M: puc19 DNA marker.

of restriction enzymes *Mva*I, *Sac*I and *Hae*III were not within Hardy-Weinberg balance. The sites of restriction enzymes *Mva*I and *Hae*III were not within Hardy-Weinberg balance, their genotypes were associated with hydatidosis resistance and susceptibility. It is not known why the sites of restriction enzymes *Sac*II and *Hae*III were within Hardy-Weinberg balance, but their genotypes were also associated with hydatidosis resistance and susceptibility.

Analysis of the relationship between *Ovar-DRB*1 genotypes and hydatidosis resistance and susceptibility in Kazakh sheep

Through statistical analysis, we found that the genotype frequencies of *Mva*Ibc, *Hin*1Iab, *Hae*IIIdd, *Hae*IIIde, *Hae*IIIdf and *Sac*IIab in control sheep were extremely significantly ($p<0.01$) higher than in hydatidosis sheep, indicating that a strong association existed between these genotypes and hydatidosis resistance. Genotype frequencies of *Mva*Ibb, *Sac*IIaa, *Hin*1Ibb and *Hae*IIIef in hydatidosis sheep were extremely significantly ($p<0.01$) higher than in controls, and genotype frequency of *Hae*IIIab was significantly higher ($p<0.05$), indicating that a significant correlation existed between these genotypes and susceptibility to hydatidosis (Table 3).

Verification by experiment of artificial hydatidosis infection

Through analyzing the haplotype resistant to genotypes, it was found that the haplotype frequency of *Mva*Ibc-*Sac*IIab-*Hin*1Iab in controls was extremely higher ($p<0.01$) than in hydatidosis sheep (Table 4), indicating that this haplotype was resistant to hydatidosis of Kazakh sheep, and this result was verified by artificial infection with hydatids. Sixteen 2-year-old Kazakh sheep which were detected as healthy by hydatidosis ELISA kit were chosen. Among them, eight with the haplotype of *Mva*Ibc-*Sac*IIab-*Hin*1Iab were taken as the treatment group, and the other eight with the haplotypes of *Sac*IIab-*Hin*1Iab or *Mva*Ibc-*Hin*1Iab or *Mva*Ibc-*Sac*IIab, which were not associated with hydatidosis resistance or susceptibility, were taken as the control. Each sheep was fed 20 adult cestodes with fertilized egg proglottis by mouth, and all 16 sheep were raised under the same conditions.

Protoscoleces can develop into cysts within 20 days after infection (Dematteis et al., 2003). The 16 sheep were slaughtered on the fourth month after hydatid infection and visual inspection of the liver and lung surfaces of each animal was made for the detection of larval stages of cestodes (Menkir et al., 2008). The results showed that there were only 2 sheep infected by hydatids in the treatment group, whereas 7 sheep were infected in the control group. Furthermore, the infection rate in the treatment group was significantly lower than in the control group ($p<0.05$) which confirmed that the genic haplotype *Mva*Ibc-*Sac*IIab-*Hin*1Iab was resistant to hydatidosis in Kazakh sheep.

DISCUSSION AND CONCLUSION

Through the technique of PCR-RFLP, the MHC-*DRB*3 (Sun et al., 2004; Yang et al., 2006) and MHC-*DRB*1 (Amills et al., 1995, 1996; Konnai et al., 2003) polymorphism of sheep and goats has been investigated and this research suggested that the MHC-*DRB* gene has extensive polymorphism. In the present study, the *Ovar-DRB*1 exon 2 of Kazakh sheep was detected by PCR-RFLP with the five restriction enzymes *Sac*II, *Mva*I, *Sac*I, *Hin*1I and *Hae*III, yielding 2, 2, 2, 2 and 6 alleles and 3, 3, 3, 3 and 16 genotypes, respectively. The polymorphism existed at the positions of the 229th, 225th, 210th, 208th, 178th, 173th, 159th, 87th base pairs. Konnai et al. (2003) studied the

Table 3. Genotype frequencies for *Ovar-DRB*1 gene in Kazakh sheep which were positive and negative (controls) for hydatidosis

Hydatidosis negative (total number is 400)			Hydatidosis positive (total number is 302)			x^2
Genotype	N	Frequency	Genotype	N	Frequency	
*Mva*Ibb	246	62.28%	*Mva*Ibb	241	79.8%	24.96**
*Mva*Ibc	149	37.72%	*Mva*Ibc	60	19.86%	25.99**
*Mva*Icc	0	0%	*Mva*Icc	1	0.33%	-
*Sac*Iaa	219	54.61%	*Sac*Iaa	142	48.14%	2.86
*Sac*Iab	142	35.41%	*Sac*Iab	115	38.98%	0.93
*Sac*Ibb	40	9.98%	*Sac*Ibb	38	12.88%	1.44
*Sac*IIaa	285	70.37%	*Sac*IIaa	243	80.46%	9.32**
*Sac*IIab	112	27.65%	*Sac*IIab	56	18.54%	7.93**
*Sac*IIbb	8	1.98%	*Sac*IIbb	3	0.99%	0.54
*Hin*1Iaa	142	35.5%	*Hin*1Iaa	109	36.09%	0.03
*Hin*1Iab	199	49.75%	*Hin*1Iab	112	37.09%	11.18**
*Hin*1Ibb	59	14.75%	*Hin*1Ibb	81	26.82%	15.71**
*Hae*IIIaa	10	2.41%	*Hae*IIIaa	15	4.97%	3.43
*Hae*IIIab	4	0.96%	*Hae*IIIab	9	2.98%	4.02*
*Hae*IIIac	3	0.72%	*Hae*IIIac	3	0.99%	0
*Hae*IIIad	3	0.72%	*Hae*IIIad	2	0.66%	0.13
*Hae*IIIae	14	3.37%	*Hae*IIIae	12	3.97%	0.19
*Hae*IIIaf	4	0.96%	*Hae*IIIaf	4	1.32%	0.01
*Hae*IIIbc	1	0.24%	*Hae*IIIbc	0	0%	-
*Hae*IIIcc	38	9.16%	*Hae*IIIcc	34	11.26%	0.88
*Hae*IIIce	34	8.19%	*Hae*IIIce	24	7.94%	0.01
*Hae*IIIcf	14	3.37%	*Hae*IIIcf	4	1.32%	2.98
*Hae*IIIdd	42	10.12%	*Hae*IIIdd	11	3.64%	10.64**
*Hae*IIIde	50	12.05%	*Hae*IIIde	9	2.98%	18.93**
*Hae*IIIdf	22	5.3%	*Hae*IIIdf	4	1.32%	7.87**
*Hae*IIIee	105	25.3%	*Hae*IIIee	96	31.79%	3.76
*Hae*IIIef	47	11.33%	*Hae*IIIef	58	19.2%	8.80**
*Hae*IIIff	24	5.78%	*Hae*IIIff	16	5.58%	0.07

Significant difference in frequency between hydatidosis positive and negative Kazakh sheep for same *Ovar-DRB*1 genotype denoted as * $p<0.05$, ** $p<0.01$.

polymorphism of the Ovar-*DRB*1 exon 2 of Suffolk sheep by PCR-RFLP with the restriction enzymes *Sac*I, *Sac*II, *Hin*1I and *Hae*III, also forming 2, 2, 2 and 6 alleles, respectively; Peng et al. (2007) studied the polymorphism of Ovar-*DRB*1 exon 2 of Chinese Merino sheep by PCR-RFLP with the restriction enzymes *Sac*I and *Hin*1I, each yielding 2 alleles and 3 genotypes. Their results were all consistent with the present study. However, with the restriction enzyme *Hae*III, 6 alleles and 15 genotypes were formed in their research, while 6 alleles and 16 genotypes were formed in our studies. This difference may be due to the different numbers and species of sheep.

In vertebrates, the MHC plays a central role in foreign antigen recognition and immune response to pathogens (Klein 1986; Hedrick et al., 1994; Bernatchez et al., 2003; Piertney et al., 2006). Some *Ovar-DRB*1 alleles may be better suited to display antigens to certain diseases and so generate a better immunity through an improved T-cell response repertoire (Krieger et al., 1991; Coppin et al., 1993). Ding et al. (2005) found that the MICA-A5·1 homozygous genotype and A5·1 allele were closely associated with ulcerative colitis and the MICA-A5·1 allele was positively associated with female ulcerative colitis patients in the Chinese population. Liu et al. (2005) studied the relationship between polymorphism of the MICA gene and the risk of oral submucous fibrosis. Schwaiger et al. (1995) and Sayers et al. (2005) found that MHC-*DRB*1 has a relationship with nematode resistance. All these results suggested that polymorphism of the MHC is closely associated with disease resistance or susceptibility. In this research, the polymorphism of *Ovar-DRB*1 exon 2 of Kazakh sheep was detected, and the frequency of genotypes were compared between hydatidosis sheep and controls by statistical methods. It was found that the *DRB*1 genotypes were significantly correlated with hydatidosis resistance and susceptibility (Table 3). After the haplotype with those

Table 4. Analysis of the relationship between different haplotype of *Ovar-DRB*1 and hydatidosis resistance

Haplotype of *Ovar-DRB*1 genes	Controls		Hydatidosis sheep		x^2
	N	Total	N	Total	
*DRB*1-*Mva*Ibc,*Sac*IIab,*Hin*1Iab, *Hae*IIIde	3	400	0	302	0.85
*DRB*1-*Mva*Ibc,*Sac*IIab,*Hin*1Iab, *Hae*IIIdd	3	400	0	302	0.85
*DRB*1-*Mva*Ibc,*Sac*IIab,*Hin*1Iab, *Hae*IIIdf	2	400	0	302	0.27
*DRB*1-*Hin*1Iab,*Sac*IIab,*Hae*IIIdd	4	400	0	302	1.53
*DRB*1-*Mva*Ibc,*Hin*1Iab,*Hae*IIIdd	4	400	0	302	1.53
*DRB*1-*Mva*Ibc,*Sac*IIab,*Hae*IIIdd	3	400	0	302	0.85
*DRB*1-*Sac*IIab,*Hin*1Iab,*Hae*IIIdf	1	400	0	302	-
*DRB*1-*Hin*1Iab,*Sac*IIab,*Hae*IIIde	2	400	0	302	0.27
*DRB*1-*Mva*Ibc,*Hin*1Iab,*Hae*IIIdf	1	400	0	302	-
*DRB*1-*Mva*Ibc,*Sac*IIab,*Hae*IIIde	3	400	1	302	0.05
*DRB*1-*Mva*Ibc,*Hin*1Iab,*Hae*IIIde	7	400	0	302	3.71
*DRB*1-*Mva*Ibc,*Sac*IIab,*Hin*1Iab	29	400	4	302	13.49**
*DRB*1-*Mva*Ibc,*Sac*IIab	13	400	13	302	0.54
*DRB*1-*Hin*1Iab,*Hae*IIIde	6	400	2	302	0.46
*DRB*1-*Hin*1Iab,*Hae*IIIdd	7	400	4	302	0.02
*DRB*1-*Sac*IIab,*Hae*IIIdd	5	400	4	302	0.06
*DRB*1-*Mva*Ibc,*Hae*IIIdd	1	400	2	302	0.06
*DRB*1-MvaIbc,*Hae*IIIde	4	400	2	302	0.00
*DRB*1-*Mva*Ibc,*Hae*IIIdf	3	400	0	302	0.85
*DRB*1-*Sac*IIab,*Hae*IIIdf	3	400	0	302	0.85
*DRB*1-*Sac*IIab,*Hae*IIIde	3	400	4	302	0.14
*DRB*1-*Hin*1Iab,*Hae*IIIde	3	400	2	302	0.1
*DRB*1-*Sac*IIab,*Hin*1Iab	12	400	18	302	3.69
*DRB*1-*Mva*Ibc,*Hin*1Iab	39	400	21	302	1.72

$X^2 > X^2_{0.01,1} = 6.63$, $p<0.01$. $X^2 > X^2_{0.05,1} = 3.84$, $p<0.05$. $X^2 < X^2_{0.05,1} = 3.84$, $p>0.01$. * p<0.05, ** p<0.01.

resistant genotypes was analyzed, the haplotype *Mva*Ibc-*Sac*IIab-*Hin*1Iab, resistant to hydatidosis, was found to exist in Kazakh sheep.

In this research, in order to verify the haplotype *Mva*Ibc-*Sac*IIab-*Hin*1Iab could be taken as a genic haplotype of hydatidosis resistance in Kazakh sheep, we adopted the method of artificial hydatid infection. Zheng et al. (2000) collected the cyst vesicle fluid from diseased livers of the sheep with hydatidosis which were artificially infected, and then injected the fluid into healthy sheep in the peritoneum. In this way, the sheep model with *Echinococcus granulosus* infection was established, whereas, in our research, adult cestodes with fertilized egg proglottis were fed orally to sheep, and the objective was to imitate natural infection with hydatids. The result of artificial hydatidosis corresponded with that of genotype frequency, indicating that the genic haplotype *Mva*Ibc-*Sac*IIab-*Hin*1Iab was resistant to hydatidosis. Therefore, *Ovar-DRB*1 genic haplotype *Mva*Ibc-*Sac*IIab-*Hin*1Iab could be used as a tagged molecule of hydatidosis resistance, and used as a molecular marker to assist in selection and breeding of new species with hydatidosis resistance in the future.

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