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RESEARCH ARTICLE

MHC-DRB1 exon 2 polymorphism and its association with mycoplasma ovipneumonia resistance or susceptibility genotypes in sheep

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Abstract. Major histocompatibility complex (MHC) polymorphisms are associated with animal and human diseases. However, only a few studies have reported an association between MHC polymorphisms and mycoplasma ovipneumonia (MO). In the present study, three resistance/susceptibility genotypes associated with MO were identified by polymerase chain reaction-restriction fragment length polymorphism genotyping, assessing the clinical and pathological features, and examining the immune factors. The current results showed that *Mva*I bb and *Hae*III ee were dominant genotypes in the susceptible Hu population, while MO-resistant populations, Dorper and D × H hybrids, were dominated by the *Mva*I cc and *Hae*III dd genotypes, suggesting that *Mva*I cc and *Hae*III dd genotypes might be associated with the trait of MO resistance. Further, the clinical symptoms and pathological morphology in the susceptibility group infected with MO were more severe than those in the resistant groups infected similarly. The data on the changes in the immune factor responses were utilized to deduce the molecular mechanism underlying the MO resistance/susceptibility. The results showed that the susceptible genotypes promote the inflammatory responses by inducing a high expression of TNFα, IFNγ, IL-4, IL-6, and IL-1β, while the resistant genotypes inhibit the inflammatory response by increasing the expression of IL-2 and IL-10 significantly. This finding would provide the theoretical guidance for propagating sheep breeds that are highly resistant to MO.

Keywords. polymorphism; genotypes; mycoplasma pneumonia; immune factors; resistance; susceptibility.

Introduction

Major histocompatibility complex (MHC) is a closely linked and highly polymorphic genetic region in the chromosome, composed of several gene loci. MHC-encoded molecules can

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recognize and present endogenous and exogenous antigens, followed by presenting the antigens to T cells, thereby leading to cellular and humoral immune responses (Millot 1978; Klein 1986). MHC polymorphism is closely related to resistance and susceptibility of many diseases, and MHC genes are considered as the primary candidates, currently rendering the phenomenon as one of the hotspots in the research of immunogenetics. In 1936, MHC genes were first shown in mice (Gorer 1937), and

this have been followed in almost all the vertebrates. Although MHC and the encoded products vary across species, the gene structure and function are similar. In addition, the MHCs in mice and rats are named as H-2 complex and RT-1 complex, respectively. While the MHC of other species are named referring to the human leukocyte antigens (HLA). For instance, the MHC in sheep is named as ovine leukocyte antigens (OLA) (Mahdy *et al.* 1989; Hediger *et al.* 1991), which is localized on chromosome 20 and divided into three classes: I, II and III. The gene region of MHC class II in sheep is similar to that of humans and is subdivided into IIa and IIb. The IIa sub-region includes loci *DRA*, *DRB1*, *DRB2*, *DRB3*, *DRB4*, *DQA1*, *DQA2*, *DQB1* and *DQB2*, and IIb includes *DYA*, *DYB*, *DNA*, *DOB*, *DMA* and *DMB* (Blattman *et al.* 1993; Escayg *et al.* 1996). The most polymorphic locus of MHC in sheep is DR (Andersson and Rask 1988), including the functional gene *DRB1* and pseudogenes *DRB2*, *DRB3* and *DRB4* (Scott *et al.* 1991). Abundant polymorphism is observed on exon 2, and the adjacent intron 2 in *DRB1*. The β1 structural domain encoded by exon 2 constitutes the DR molecular peptide-binding region, which regulates the immune responses. It presents exogenous antigens to helper T-lymphocytes (Th, CD4+) to trigger the immune response, and hence, serves as a candidate gene for immune response and disease resistance (Brown *et al.* 1993; Palti *et al.* 2001). Currently, the reports on sheep MHC gene resistance/susceptibility primarily focus on class II, especially the polymorphism on exon 2 in the *DRB1* gene (Schwaiger *et al.* 1995; Paterson *et al.* 1998; Sayers *et al.* 2005; Dukkipati *et al.* 2010). GenBank database has included >120 amino acid polymorphic sequences of *DRB1* exon 2 encoding the β1 structural domain (Dutia *et al.* 1994; Jugo and Vicario 2001; Hemrrann *et al.* 2005). Also, a number of studies have reported the correlation between MHC in sheep breeds and disease (Ballingall and Riccardo 2010; Li *et al.* 2010, 2011a; Hui *et al.* 2012; Shen *et al.* 2014). However, none of the studies have yet addressed the correlation between sheep *MHC-DRB1* gene polymorphism and mycoplasma pneumonia (MP) resistance.

MP, also known as mycoplasma pleuropneumonia, is a chronic respiratory tract infectious disease mainly caused by *Mycoplasma ovipneumoniae* (MO), manifested as cough, tachypnea, exercise intolerance, gasp, nasal discharge, progressive emaciation, and proliferative inflammation in pulmonary interstitium. The disease cannot be cured easily, and shows a high incidence among lambs. The sick lambs present weight loss, and the fatality rate is higher than that of the healthy adult sheep. The feed conversion ratio of the sick sheep is low, incurring a high medical cost, that in turn causes a huge economic burden to the sheep industry worldwide (Ayling and Nicholas 2007; Bottinelli *et al.* 2017). Xinjiang is a region with high incidence of the disease, and the loss accounts for >20% of all the diseases in sheep and goat breeding industry (Song *et al.* 2011). Occupants of Uyghurs ethnicity with large demand for mutton dominated this region. Since the availability of mutton in this area is low, several high-fecundity and meat sheep breeds has been introduced to improve the local breed

in recent years. The epidemiological investigations of the new breeds have proved that high-fecundity Hu sheep is susceptible to MP (Li *et al.* 2005; Tao *et al.* 2006); especially, the infection rate of lamb is 40–60%, and the fatality rate is 10–20%. The Dorper sheep with superior meat quality is resistant to the disease; also, the hybrid generation is resistant to disease similar to the male parent (Li *et al.* 2005; Tao *et al.* 2006, 2007; Song *et al.* 2011). Interestingly, Dorper and Hu sheep are introduced from other places. However, after introduction into Xinjiang, these breeds showed a large differential resistance to MO, laying the foundation for the investigation of MO resistance in different sheep breeds.

Thus, in this study, the polymorphism of exon 2 in *MHC-DRB1* among the three sheep groups was analysed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) genotyping. Also, the correlation between the genotype and resistance/susceptibility to MP was studied. The artificial MO-infection experiment was used to detect the disease resistance capability of sheep with candidate genotypes and explore the molecular mechanism, which provided a theoretical basis and reference for the application of anti-disease genotype of MHC class II gene in breeding to gain disease resistance.

Methods and methods

Animals and sample preparation

The blood samples were collected from 400 Hu sheep in Xinbao sheep farm, 560 Dorpers in Rifa sheep farm, and 255 F_1 generations in Xinbao and Rifa sheep farm of Aksu, Xinjiang. A volume of 10 mL blood sample was withdrawn from each sheep, of which, 5 mL with anticoagulant was used to extract the genomic DNA, and the remaining was used to obtain serum at room temperature for further detection of the rate of MO.

PCR of MHC-DRB1 exon 2

The primer sequence was synthesized by Sangon Biotech (Shanghai) as described previously (Konnai *et al.* 2003). The 20 μL reaction system comprised of 1 μL of genome DNA at 150 ng/μL, 10 μL of 2× PCR mix, and 0.5 μL of upstream and downstream primers (100 μM) each. The amplification conditions were as follows: predenaturation for 5 min at 94°C; 35 cycles at 94°C for 30 s, 63°C for 30 s, 72°C for 30 s; 72°C for 7 min.

RFLP genotyping

*Sca*I, *Hae*III, *Sac*II, *Mva*I and *Bsa*HI restriction endonucleases (table 1) were used to digest the products. The 20 μL reaction volume consisting of 5 μL of PCR product, 1 μL of

10× buffer, and 0.5 μL of 10 U/μL endonuclease was incubated at 37°C for 4 h or overnight. The cleaved products were detected using agarose gel electrophoresis and typed using standard restriction map provided by Konnai (Konnai *et al.* 2003).

MO indirect hemagglutination assay

The MO detection method previously described (Cheng *et al.* 2015) was used to detect the MO infection in sheep serum. Briefly, 96 V-shaped well plate was added with 25 μL of dilution buffer from the first to eight wells. In first well, 25 μL of the serum samples was added and mixed well. Twenty-five microliters of the mixture was add to the second well. The dilution continued until the eighth well. The plates were shaken for 2 min and then incubated at 37°C for 2 h without shaking. The test was considered positive when a layer of agglutinated erythrocytes was formed in wells at dilutions of 1:16 or higher, and positive and negative controls were included in each test. The positive rate of the infection was analysed in 100 samples.

MO experimental infection experiment in standard sheep

In total, 15 healthy with antibody negative, similar ages, body weights hybrid genotype F_1 male sheep (similar type and weight) with *Hae*III dd, *Mva*I cc and *Hae*III ee were randomly selected from 255 F_1 generations in Xinbao and Rifa sheep farm and divided into groups A, B and C, respectively ($n = 5$). One day before the experimental infection (-1 day), experimental infection (0 day) and on days 3, 7, 14, 21 and 27 after infection, two samples of fresh nonanticoagulant blood were collected at seven time points. At day 0, 5 mL of sheep MO was input into the trachea of the animal in the experimental groups as Y-98 standard strain culture medium (containing CFU = 10^6). The lung tissues infected with MO after death and 27 days after the experiment were collected for histopathological observation.

Observation and scoring of pathological change in lung tissue

The clinical manifestations in lung tissues were observed, and the pathological change was scored based on the clinical symptoms and evaluation criteria proposed by Nicholas *et al.* (2002). Sheep were scored for mental state, respiratory symptoms, rogue, severity and duration of cough and arthritic lesions. Details of the clinical assessment are given in table 1 in electronic supplementary material at http://www.ias.ac.in/jgenet/.

Preparation of histopathological sections and pathological scoring

Lung tissue samples were immersed in 10% formaldehyde, dehydrated, cleared, embedded, consecutively sliced (thickness 5–7 μm), unfolded, dried, stained with haematoxylineosin, and observed under an optical microscope to describe the pathological characteristics. The pathological status of the experimental sheep was scored according to the MP histopathological standard in Golden Syrian Hamster (Cimolai *et al.* 1992). The histopathologist was blinded to the source of tissue and, the right and left lung sections from sheep were randomized within the entire set of slides to be examined. The pathology grading system consisted of a numerical score ranging from 0 to 26 (table 2 in electronic supplementary material). Each sagittal section was scored based on a cumulative total from five categories that incorporated an evaluation of: (i) number of bronchiolar and bronchial sites involved by the periluminal infiltrate, (ii) severity of periluminal infiltrate, (iii) severity of luminal exudate, (iv) frequency of perivascular infiltrate, and (v) severity of parenchymal pneumonia. The scores from each lung section were added and then divided by two to arrive at a score per sheep.

Detection of cytokines by ELISA

The sera were collected from experimental sheep before and after infection, and the change in the level of cytokines

Table 1. The genotypes of PCR-RFLP by restriction enzyme *Hae*III in exon 2 of the *MHC-DRB1* gene.

Genotypes	Restriction fragments (bp)	Genotypes	Restriction fragments (bp)
*Hae*III AA	173 + 71 + 48 + 4	*Hae*III AF	173 + 159 + 71 + 52 + 48 + 14 + 4
*Hae*III BB	173 + 123	*Hae*III BD	173 + 159 + 123 + 14
*Hae*III CC	159 + 137	*Hae*III BE	173 + 159 + 123 + 71 + 66
*Hae*III DD	159 + 123 + 14	*Hae*III CD	159 + 137 + 123 + 14
*Hae*III EE	159 + 71 + 66	*Hae*III CE	159 + 137 + 71 + 66
*Hae*III FF	159 + 71 + 52 + 14	*Hae*III CF	159 + 137 + 71 + 52 + 14
*Hae*III AB	173 + 123 + 71 + 48 + 4	*Hae*III DE	159 + 123 + 71 + 66 + 14
*Hae*III AC	173 + 159 + 137 + 71 + 48 + 4	*Hae*III DF	159 + 123 + 71 + 52 + 14
*Hae*III AD	173 + 159 + 123 + 71 + 48 + 4	*Hae*III EF	159 + 71 + 66 + 52 + 14
*Hae*III AE	173 + 159 + 71 + 66 + 48 + 4		

(TNFα, IFNγ, IL-1β, IL-2IL-4, IL-6, IL-10) was detected by ELISA kits. All the specific ELISA kits were purchased from BlueGene (Shanghai, China).

Statistical analysis

The correlation between the genotype frequency and MP was analysed by chi-square test. Hardy–Weinberg equilibrium analysis was also performed by chi-square test. Other data were analysed by one-way ANOVA using SPSS 17.0 software. The data were expressed as mean ± SD. $P < 0.05$ and $P < 0.01$ indicated significant difference.

Results

PCR-RFLP genotyping of MHC-DRB1 exon 2 gene

OLA-ERB1 and OLA-HL031 primers were used to amplify the exon 2 of the *MHC-DRB1* gene in the three sheep groups that provided the products of 296 bp.

*Sac*I, *Sac*II, *Mva*I, *Bsa*HI and *Hae*III restriction endonucleases were used to cleave the amplified products of exon 2 in the *MHC-DRB1* gene from 560 Dorper sheep, 400 Hu sheep and 255 D × H F$_1$ hybrids. The polymorphic variations could be detected in the exon 2 of *DRB1* gene in the three sheep groups using the specific restriction endonucleases mentioned above. The cleavage of *Sac*I, *Sac*II, *Mva*I and *Bsa*HI was controlled by bialleles, representing three genotypes respectively (figure 1, a, c&d). By contrast, *Hae*III restriction endonuclease exhibited a great difference among the three sheep groups (figure 1b), the PCR products showed three genotypes after *Mva*I, *Sac*I, *Sac*II and *Bsa*HI endonuclease digestion, and the PCR product *Hae*III

endonuclease can produce up to 15 genotypes. The size of the digested fragments are shown in tables 1 and 2.

Correlation between polymorphism of exon 2 in MHC-DRB1 and resistance/susceptibility of MP

To explore the correlation between *MHC-DRB1* gene polymorphism and the trait of MP resistance or susceptibility, the genotypes from the MO-susceptible sheep population (Hu sheep) and MO-resistant populations (Dorper sheep and D × H F$_1$ hybrids) were analysed. Table 3 summarizes the distribution of genotypes and frequencies. Hu sheep with a high risk of MP were used as control, while the others were experimental sheep groups. The results showed that genotype percentage of *Hae*III ee and *Mva*I bb in the Hu sheep was higher than that of the experimental groups, while genotypes in terms of *Hae*III dd and *Mva*I cc occurred more often in the Dorper sheep when compared with the control group (table 3). Further, significant frequency differences were observed in *Hae*III ee, *Mva*I bb, *Hae*III dd and *Mva*I cc genotypes between the control and experimental groups ($P < 0.001$). *Mva*I bb genotype was dominant with a frequency of 70% in the Hu sheep group, and the allele frequency of *Mva*I B was up to 80% (table 3 in electronic supplementary material). The frequencies of the other four dominant genotypes, *Sac*I aa, *Sac*II aa, *Bsa*HI bb, and *Hae*III ee, were >50%. Intriguingly, the trend of *Sac*I aa, *Sac*II aa, and *Bsa*HI bb genotypes frequency in Dorper sheep was same as that of Hu sheep (table 4 in electronic supplementary material). However, *Mva*I and *Hae*III restriction endonuclease cleavage sites differed markedly. The *Mva*I cc genotype frequency in Dorper was up to 60% but only 10% in the Hu sheep. The *Hae*III dd genotype frequency in Dorper was up to 48% but was only 20% in the Hu sheep.
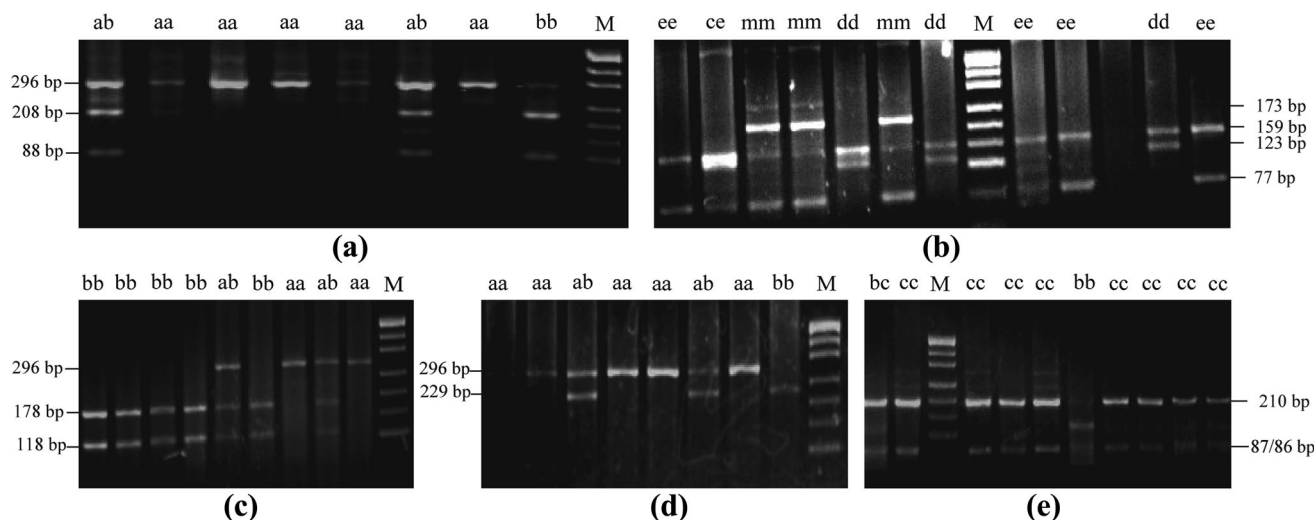

Figure 1. PCR-RFLP genotyping of five endonucleases. (a)–(e) Represent the electrophoretic patterns of the second exon of *MHC-DRB1* digested with *Sac*I, *Hae*III, *Bsa*HI, *Sac*II and *Mva*I, respectively. The lanes marked with the corresponding genotypes. M, pUC19 DNA marker.

Table 2. The genotypes of PCR-RFLP by restriction enzymes in exon 2 of the *MHC-DRB1* gene.

Restriction enzymes	Genotypes of each restriction enzyme		
*Sac*I	aa (296 bp)	ab (296 bp / 208 bp / 88 bp)	bb (208 bp / 88 bp)
*Bsa*HI	aa (296 bp)	ab (296 bp / 178 bp / 118 bp)	bb (178 bp / 118 bp)
*Mva*I	bb (123 bp / 87 bp / 86 bp)	bc (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)

E-cut fragments are in parenthesis.

Table 3. The frequencies of genotypes and alleles in *MHC-DRB1* exon 2 of the three groups.

Genotypes	Genotypic frequencies (*n*)		
	Hu sheep	Dorper sheep	DH-F$_1$ sheep
***Mva*I bb**	0.7000 (280)	0.2250 (126)***	0.2471 (63)***
*Mva*I bc	0.2000 (80)	0.1786 (100)*	0.1372 (35)*
***Mva*I cc**	0.1000 (40)	0.5964 (334)***	0.6157 (157)***
*Sac*II aa	0.6750 (270)	0.8214 (460)**	0.5294 (135)*
*Sac*II ab	0.2250 (90)	0.0822 (46)*	0.2431 (62)
*Sac*II bb	0.1000 (40)	0.0964 (54)	0.2375 (58)*
*Sac*I aa	0.6200 (248)	0.9179 (514)**	0.8196 (209)**
*Sac*I ab	0.2775 (111)	0.0786 (44)**	0.1373 (35)*
*Sac*I bb	0.1025 (41)	0.0035 (2)**	0.0431 (11)*
*Bsa*HI aa	0.1875 (75)	0.3536 (198)**	0.2863 (73)*
*Bsa*HI ab	0.3425 (137)	0.2321 (130)*	0.1412 (36)**
*Bsa*HI bb	0.4700 (188)	0.4143 (232)	0.5725 (146)*
*Hae*III aa	0.0100 (4)	0.0000 (0)	0.0314 (8)
*Hae*II bb	0.0050 (2)	0.0179 (10)*	0.0314 (8)**
*Hae*III cc	0.0375 (15)	0.0214 (12)	0.0157 (4)*
***Hae*III dd**	0.2050 (82)	0.4821 (270)***	0.5804 (148)***
***Hae*III ee**	0.5250 (210)	0.2321 (130)***	0.1412 (36)***
*Hae*III ab	0.0050 (2)	0.0000 (0)	0.0000 (0)
*Hae*III ac	0.0050 (2)	0.0000 (0)	0.0000 (0)
*Hae*III ae	0.0075 (3)	0.0000 (0)	0.0000 (0)
*Hae*III bd	0.0000 (0)	0.0036 (2)	0.0000 (0)
*Hae*III be	0.0075 (3)	0.0071 (4)	0.0235 (6)**
*Hae*III ce	0.0200 (8)	0.0071 (4)**	0.0078 (2)**
*Hae*III cd	0.0000 (0)	0.0071 (4)	0.0157 (4)
*Hae*III cf	0.0000 (0)	0.0036 (2)	0.0000 (0)
*Hae*III de	0.1225 (49)	0.0786 (44)*	0.0510 (13)*
*Hae*III df	0.0025 (1)	0.0107 (6)*	0.0080 (1)*
*Hae*III md	0.0050 (2)	0.0286 (16)**	0.0667 (17)**
*Hae*III mm	0.0200 (8)	0.0750 (42)**	0.0353 (9)*
*Hae*III me	0.0225 (9)	0.0250 (14)	0.0000 (0)

The same genotypes in the control group (Hu sheep) and the test group (Dorper and DH-F$_1$ sheep), respectively. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

Conversely, *Hae*III ee genotype frequency in Hu sheep was up to 52.5% but was only 23% in the Dorper group.

To further confirm the correlation between MO-resistant/susceptible genotype and MP in sheep, a total of 100/1115 blood samples were randomly selected to detect the mycoplasma antibody level; of which, 21 were positive for MO. The ratios of *Hae*III ee, *Mva*I bb, *Hae*III dd and *Mva*I cc genotypes in the 21 blood samples were analysed, and the ratios of MO-resistant *Mva*I cc and *Hae*III dd genotypes were lower than the theoretical genotype frequency. Moreover, the ratios of MO-susceptible *Hae*III ee and *Mva*I bb genotypes were higher than the theoretical genotype frequency (figure 2). The frequency of the MO-resistant *Mva*I cc and *Hae*III dd differed significantly from that of MO-susceptible *Hae*III ee and *Mva*I bb genotypes ($P < 0.05$).

Comparison of experimental infection of MP and infection effect between MO-resistant and MO-susceptible genotypes

Isolation and diagnosis of mycoplasma after experimental infection: Smears were prepared from lung tissues samples obtained from deceased sheep that were stained and examined by microscopy. Further, after incubation at 37°C for two days, any pathogenic micro-organisms were not detected on the normal agar, blood agar and MacConkey agar plates, and the colour of the mycoplasma-specific culture medium was not altered. Subsequently, after incubation for seven days under 5% CO_2, the colour of the medium changed from pink to yellow. The suspected mycoplasma culture was smeared on the cover glass and stained with Giemsa, which revealed spherical, ring, and multishaped mycoplasma by microscopic examination. The mycoplasma was seeded in a plate and incubated at 37°C in the presence of 5% CO_2 for four days. The colony with typical mycoplasma morphology, as observed under the microscope (figure 3), indicated successful experimental infection.

Observation and scoring of clinical manifestation: Any significant clinical manifestations were not observed in the three groups after seven days post-MO infection. However, after 16 days, two sheep in group C presented clinical manifestations, such as anorexia, diarrhea, emaciation, light cough, serous nasal or purulent nasal, astasia, and severe symptoms such as dyspnea and recumbency. The two sheep perished on days 18 and 25, respectively. Table 4 describes the clinical manifestation sum scores of the three groups, and the score of clinical manifestation in the group C was significantly higher than that in the groups A and B ($P < 0.05$); however, no significant difference was found between groups A and B ($P > 0.05$) (table 4).

Pathological observation and score of lung tissue: The changes in the histopathological sections of the lung tissues from the
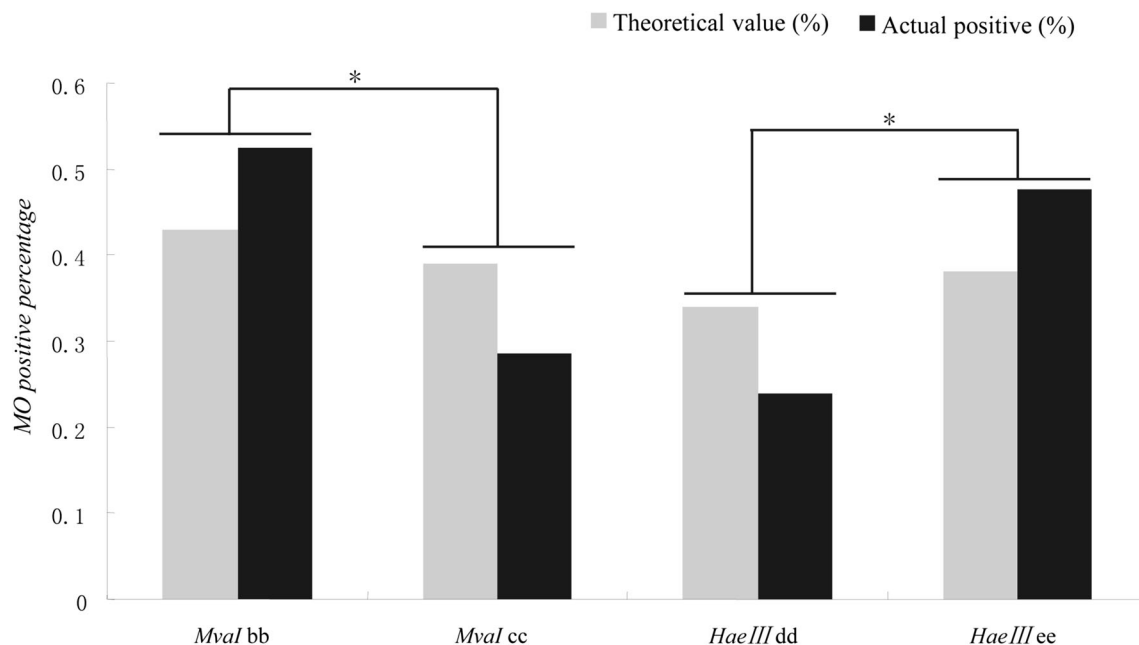
Figure 2. Percentage of candidate susceptibility and resistant genotypes among 21 MO antibody positive animals. Gray colour, theoretical value; black colour, means actual positive; *$P < 0.05$.
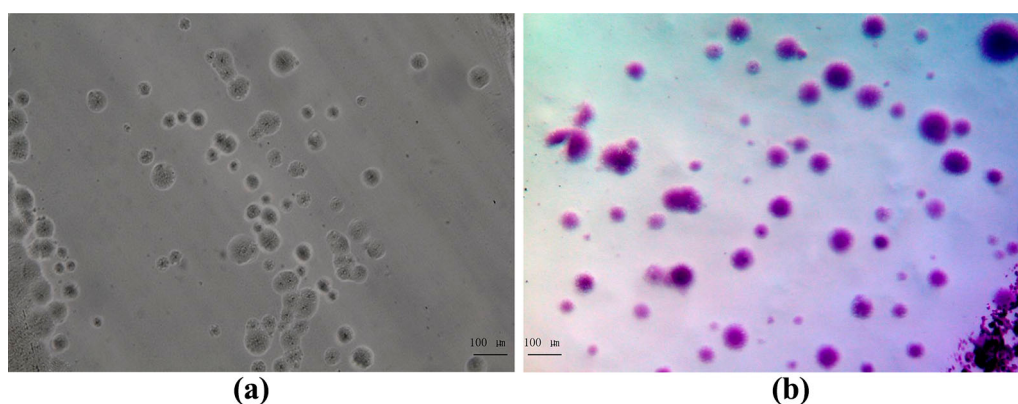

Figure 3. Morphology of MO (a) MO colony under a light microscope (40×); (b) MO colony with dienes staining under a light microscope (40×).

sheep deceased due to sickness and slaughter were observed and scored. As shown in figure 4, the lung was infected in different degrees in the three groups. The lesion in the MO-susceptible group C showed maximal pleural effusion. The MO infection altered the serous fibrous pleural pneumonia, massive pleural effusion, pulmonary pleural fibrosis, pink consolidation in different degrees on the lung surface, haemorrhagic spot, interstitial broadening, and marble-like section in the pneumonic patch due to dry and interstitial edema (figure 4). The degree of injury, lung colour, adhesion between lung tissues and thoracic cavity, and pleural effusion were used as indicators to score the lung tissues in the MO-infected sheep. The pathological change score of group C was significantly higher than that in groups A and B ($P < 0.01$). However, no significant difference was observed between groups A and B ($P > 0.05$) (table 5).

The pathological changes in the experimental groups were further scored based on the slices. The average values in groups A and B were 9.2 and 10.2, respectively. The pathological lesion was light, representing the broadening between the alveolar and pulmonary interstitium. A small amount of lymphocytic infiltration was observed in the mesothelium, while a small amount of inflammatory cell infiltration was observed in the lumen. The alveolar wall was expanded by the capillary blood, and the red blood cells were dissolved in individual vessels (figure 5). The average value in group C was 21.8, which is significantly higher than that of groups A and B ($P < 0.01$) (table 6), thereby indicating a severe pathological change. This phenomenon indicated that the lesion was wide with a large area, the pulmonary interstitium was significantly broadened, a large amount of lymphocytic infiltration was observed in the

Table 4. Score of clinical symptoms in the three groups of sheep.

Checklist	Resistant A group	Resistant B group	Susceptible C group
Mental state	2	2	9
Respiratory symptoms	1	0	9
Rogue	3	4	7
Cough severity	2	1	6
Arthritis	2	1	9
Pleurisy	3	2	8
Excrement	0	0	6
Total scores	13[a]	10[a]	54[b]

The same lowercase letter indicates no significant difference between the groups ($P > 0.05$). Different lowercase letters indicate significant differences between the groups ($P < 0.05$).

Table 5. Scores of pathological changes in the lung tissue in the three groups.

Checklist	Resistant A group	Resistant B group	Susceptible C group
Degree of lung injury	4	5	11
Lung colour	3	4	11
Pleural adhesions	3	3	8
Pleural effusion	3	3	10
Total scores	13[a]	15[a]	40[b]

The same lowercase letters indicate no significant difference between the groups ($P > 0.05$). Different lowercase letters indicate significant differences between the groups ($P < 0.01$).

septum, the bronchial epithelial structure was damaged or fell off, and significant number of lymphocytes exuded in the lumen of the bronchiole accompanied by congestion in the blood capillary of pulmonary interstitium and venule near the bronchus. Compared to the infected sheep alveoli, the alveolar score in the normal group was <1 without any significant pathological change.
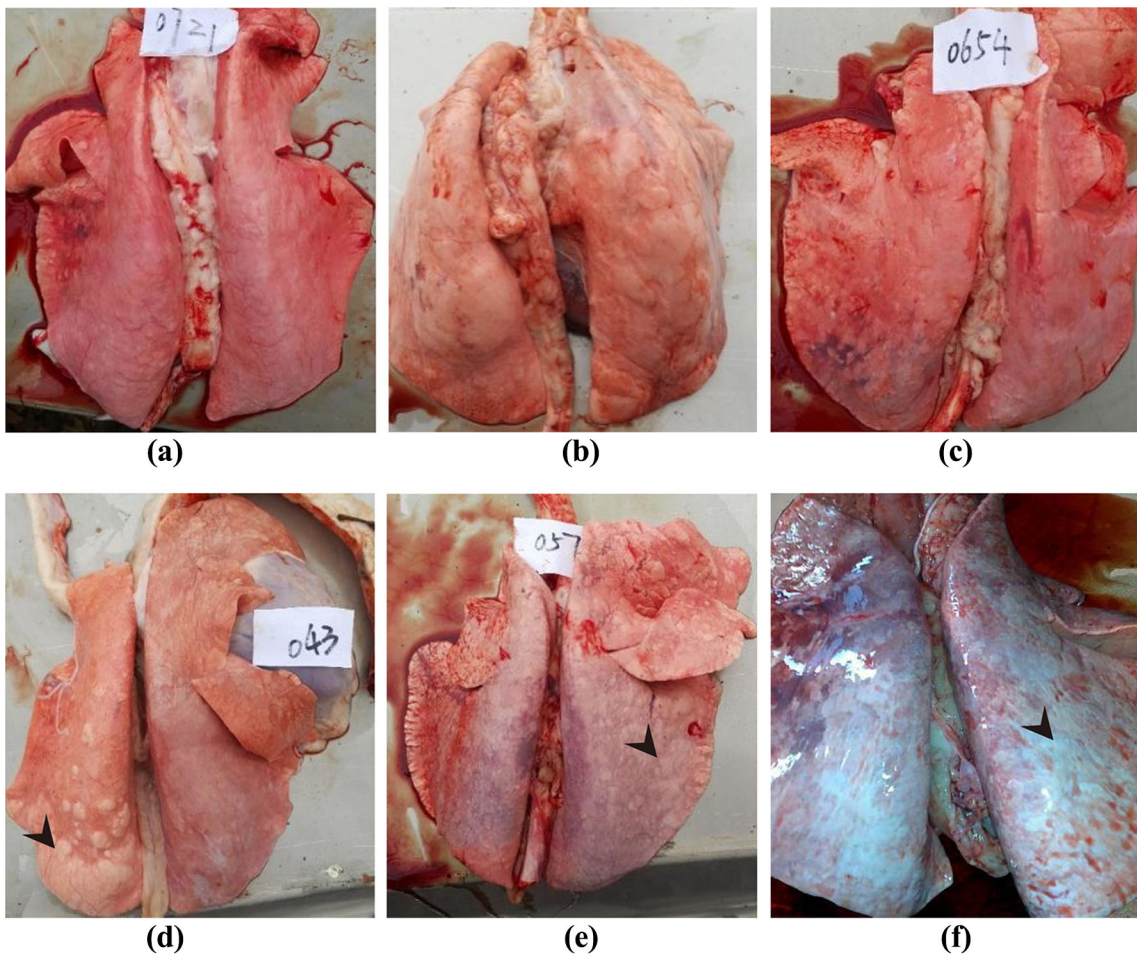

Figure 4. Pathological changes in the lung of sheep infected with MO. (a)–(c) Represents the pathological changes in the infected lung in a subset of samples in resistance groups (a&b). (d)–(f) Represents pathological changes in the infected lung in a subset of samples in the susceptibility group (c). Arrows indicate the marble-like surface changes.
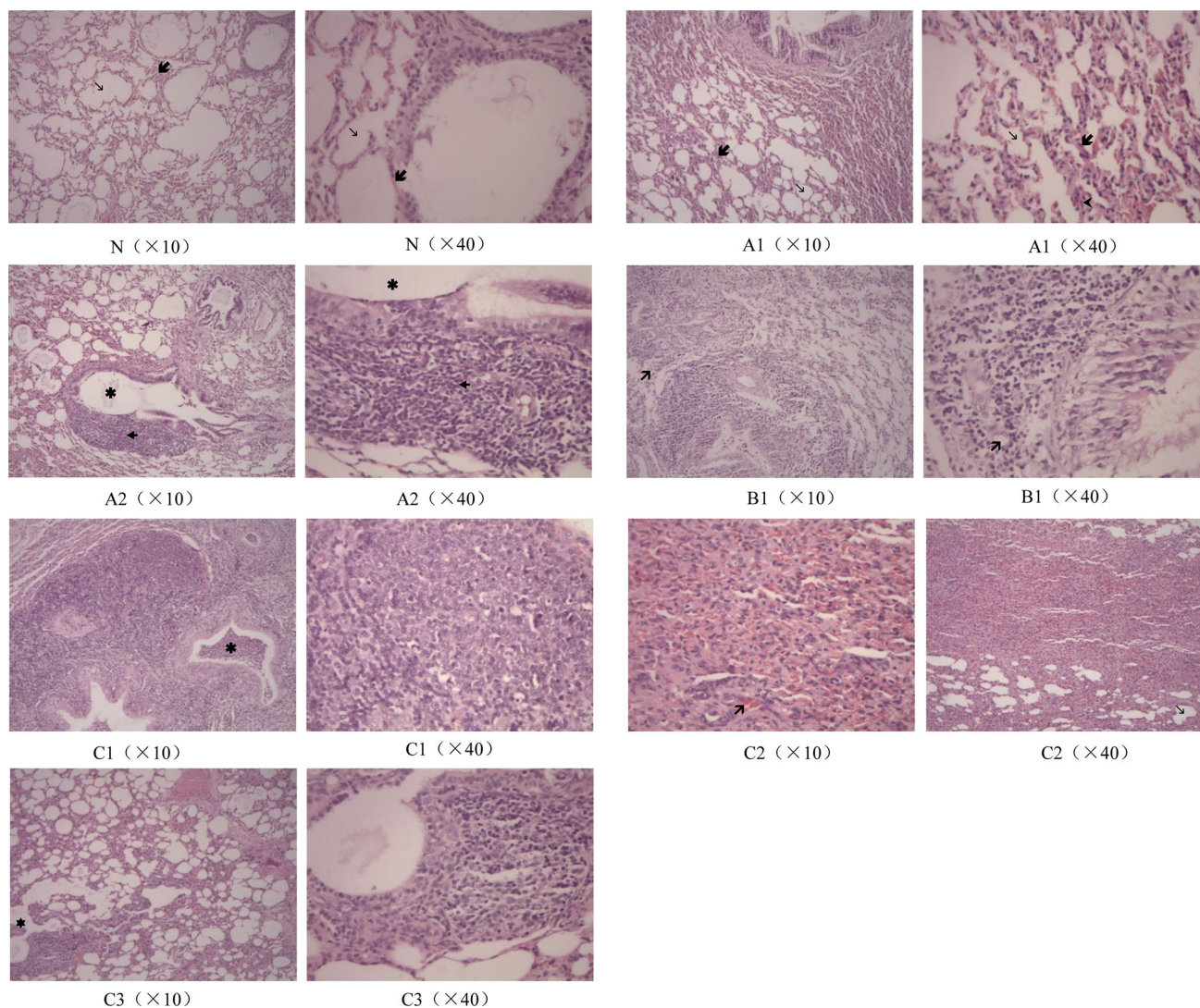
Figure 5. Pathological morphology of sheep infected with MO. N, normal lung without infection; A, B, and C, MO-infected lung of A, B, and C groups, respectively. N, normal alveolar (↘) and alveolar interstitial (↙); A1, alveolar (↘) and widened alveolar interstitial (↙), alveolar wall with capillary blood is highly dilated and filled with red blood cells (◄); A2, multiple lymphocytes (◄) infiltrate on the side of the small bronchi (✱); B1, the structure of the lung tissue is controversial. Inflammatory cells (lymphocyte infiltration) are observed in the respiratory bronchus (↗). C1, the structure of the lungs disappeared, a large number of lymphocytes infiltrated around the small bronchioles (✱) in the lungs, lymphocytes infiltrated in the small bronchi, and necrotic foci were observed. C2, alveolar (↘) is reduced, alveolar wall capillary blood is highly dilated (↗), and erythrocytosis is increased; C3, multiple lymphocytes infiltrating on the side of the respiratory bronchioles (✱), and red blood cells are dissolved in individual blood vessels.

Altered expression level of serum cytokines before and after artificial MO infection: To explore the immune response of MO-resistant and MO-susceptible genotypes before and after MO-infection, the cytokines response in groups A (*Hae*III dd genotype), B (*Mva*I cc genotype) and C (*Hae*III ee genotype) was detected. The expression of TNF-α, IFN-γ, IL-1β, and IL-2 increased initially followed by a decrease. The peak times of cytokine secretion in groups A and B were earlier than that of group C. The expression levels of TNF-α, IFN-γ, and IL-1β in group C were higher than those of groups A and B, differing significantly after 14 days and markedly at some point of time. The expression level of IL-2 in group C was lower than that of groups A and B, reaching a significant difference at day seven postinfection and an extremely significant difference at days 14 and 21. At day 27, the trend returned to baseline. The humoral cytokines IL-4, IL-6 and IL-10 before and after MO infection showed an increasing trend, followed by a decrease, and the amplitude of change was significant with a shorter peak time as compared to the cellular cytokines (TNF-α, IFN-γ, IL-1β). In addition, the expression levels of IL-4 and IL-6 in groups A and B were lower than those of group C, and the difference became significant at day three after experimental infection. The content of IL-10 in group C was high, which was reduced subsequently as compared to that of groups A and B (figure 6).

Table 6. Pathological scores of the lungs in the three groups of sheep.

Checklist	Resistant A group	Resistant B group	Susceptible C group
Total scores	9.2^a	10.2^a	21.8^b

The same lowercase letters indicate no significant difference between the groups ($P > 0.05$). Different lowercase letters indicate significant differences between the groups ($P < 0.01$).

Discussion

MHC plays a key role in recognition of adaptive immunity and antigen presentation. The high polymorphism is conducive to the expansion of presentation range of antigen in the animal population and the survival and continuation of human and animals, which has been widely proved in mammals (Ammer *et al.* 1992; Ujvari and Belov 2011; La Gruta *et al.* 2018; Trier *et al.* 2018). Some mutations are associated with the diseases of human and animals (Daly and Day 2001; Shiina *et al.* 2004; Liu *et al.* 2004; Li *et al.*
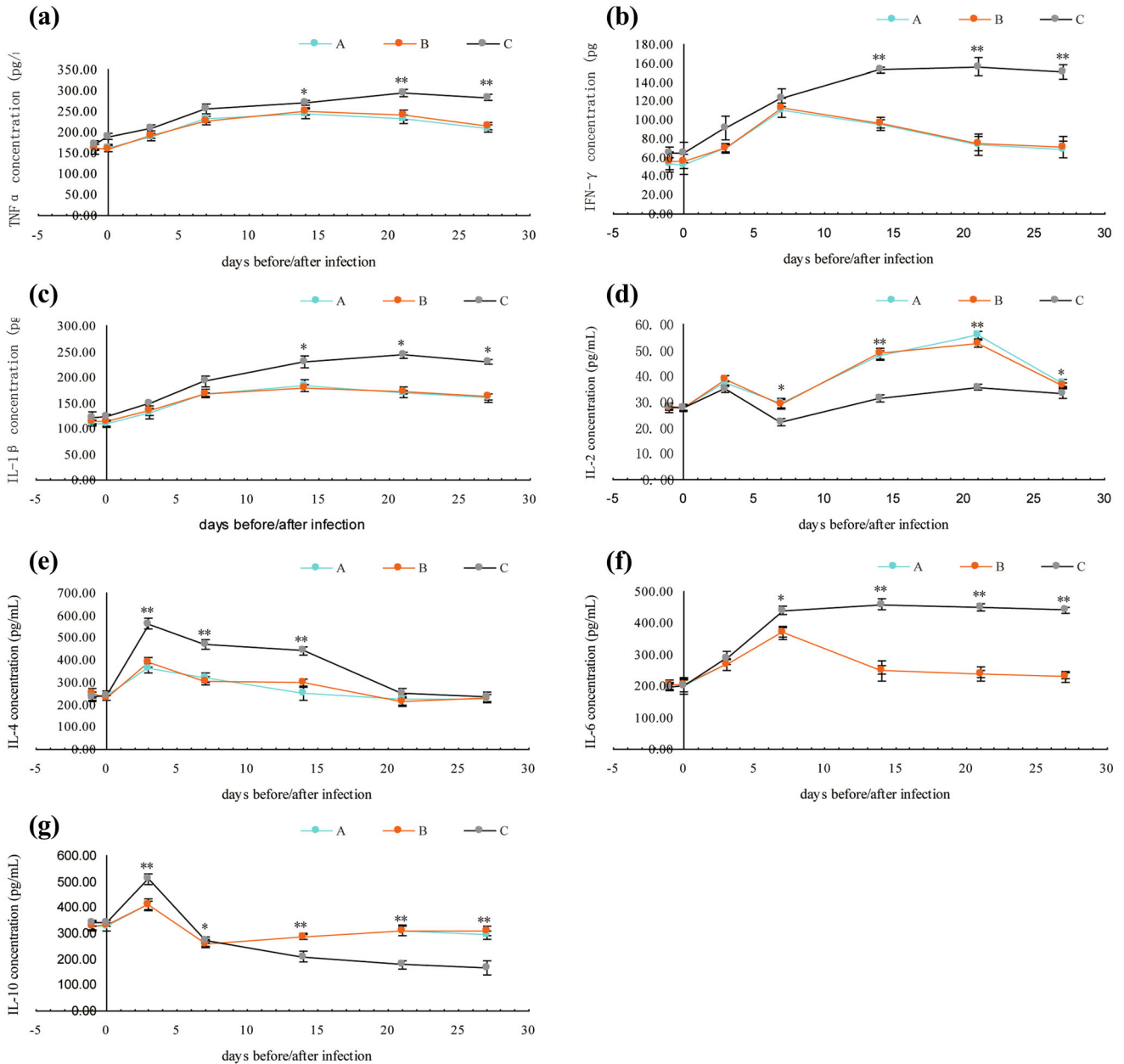

Figure 6. The expression level of serum cytokines. (a), TNF-α; (b), IFN-γ; (c), IL-1β; (d), IL-2; (e), IL-4; (f), IL-6; (g), IL-10. **A & B**, resistance groups; **C**, control susceptibility group.

2011a, b). In this study, we found that the frequency of *Hae*III ee and *Mva*I bb genotypes in Hu sheep was significantly higher than the Dorper with strong MO resistance. On the other hand, the *Hae*III dd and *Mva*I cc genotypes were commonly observed in Dorper, suggesting that the *Hae*III ee and *Mva*I bb are susceptible genotypes of MP, while *Hae*III dd and *Mva*I cc were the resistant genotypes. Next, all the typed samples were randomly sampling inspected to detect the positive rate of MO antibody. These results proved that the frequency of *Hae*III dd and *Mva*I cc was lower than the infection rate of susceptible genotypes, further indicating that *Hae*III dd and *Mva*I cc could resist the MO infection. The MHC gene polymorphism is correlated with the resistance/susceptibility of the disease, which is currently the research hotspot in the field of molecular breeding for disease resistance in livestock. Hitherto, the susceptibility of a large amount of bacteria, virus and parasite infectious diseases has been found to be correlated with MHC, e.g. *Herpes* virus, *Rous sarcoma* virus (Briles *et al.* 1977; Longenecker and Gallatin 1978; Schierman and Collins 1987; Kaufman and Venugopal 1998; Mejdell *et al.* 1994) in chicken, mastitis, leukaemia, intestinal parasite and tick in cows (Aarestrup *et al.* 1995; Sharif *et al.* 1998), *Pasteurella multocida*, diarrhea in piglet, and hereditary malignant skin melanoma and colibacillosis in pigs (Tissot *et al.* 1989; Renard *et al.* 2001). Further, the sheep carrying *DRB1* with allele 70–71 encoding Arg–Lys resisted the infection of bovine leucosis virus (BLV) and inhibited the tumour development (Nagaoka *et al.* 1999). In 2003, Konnai *et al.* (2003) found that the sheep with different exon 2 genotypes differed markedly in the expression of immune response factors after BLV infection, which was proposed as the molecular mechanism underlying BLV resistance in sheep. Sayers *et al.* (2005) found that *MHC-DRB1* genotype exerted genetic resistance in Suffolk and Poll Dorset that was linked with nematode infection. In addition, the sheep MHC polymorphism was closely related to the development and incidence of echinococcosis (Gottstein and Bettens 1994; Eiermann *et al.* 1998; Godot *et al.* 2000; Li *et al.* 2011b; Shen *et al.* 2014). To the best of our knowledge, the present study, for the first time, reported the strong correlation between *MHC-DRB1* gene polymorphism and sheep MP.

The Hu sheep with a high risk of MP differed in the breed, size and genetic characteristics as compared to Dorper with low risk. However, the F_1 generation with a low incidence of MP shared genetic characteristics with their parents, such that the genetic background was relatively consistent. Thus, the F_1 generation was the optimal group for investigating the susceptibility of MP and resistance gene. *Hae*III dd and *Mva*I cc genotypes with resistance and *Hae*III ee genotype with susceptibility in the F_1 generation were selected for artificial MO-infection. The clinical manifestations and histopathological scores indicated that MO anti-infection of *Hae*III dd and *Mva*I cc genotypes was significantly stronger than that of *Hae*III ee. The resistant group did not show any severe symptoms of

MP, such as severe cough, asthma, high temperature after MO infection, rather only light cough and exudation of a small amount of sticky secretions from the nasal cavity were observed. Subsequently, in the MP-susceptible group, severe infection symptoms were noted over a period, and two sheep deceased during the infection period. The score of pnaeumonia degree in the MO-susceptible group was significantly higher than that in the high-resistance group. Cimolai used MP to infect golden Syrian hamster to establish a pnaeumonia model and a histopathological scoring system of MP (Cimolai *et al.* 1992). Zhang *et al.* (2012) used the scoring system for evaluating the histopathology of sheep MP. This evaluation system could objectively evaluate the grade of pnaeumonia severity. In addition, the pulmonary inflammatory response and pathological change in the lung were included in the evaluation indices, and the final score objectively evaluated the severity of pnaeumonia. Thus, this scoring system was used in the present study, and the obtained histopathological scores of resistant/susceptible sheep were consistent with the pathological scores, which was in line with the previous studies (Turner 1996; Zhang *et al.* 2012).

Reportedly (Gottstein and Hemphill 1997; Rigano *et al.* 2003), mycoplasma infection can produce various cellular immune factors that bind to receptors on the cell surface, mediating and regulating the immune function and inflammatory response. Some studies demonstrated that these cell immune factors had complicated correlations among each other. For example, these proteins interacted with each other and influenced the formation of various immune globulins, complements and acute phase reactive protein, thereby building a complex network system (Torcal *et al.* 1996; Zhang *et al.* 2003). In the current study, the expression levels of serum TNFα, IFNγ, IL-1β, IL-2, IL-4, IL-6 and IL-10 in the sheep infected with MO were initially increased and then decreased, suggesting that various inflammatory cytokines participated in the occurrence and development of MP, resulting in immune function disorder. After infection, the cytokines TNFα, IFNγ, IL-4, IL-6, and IL-1β, which initiate the inflammatory response or promote an inflammatory response in the susceptible group were rapidly activated, and the expression was significantly higher in the middle and late stages of infection as compared to that of the resistant groups. Further, the levels of IL-2 and IL-10, which promote the immune response of the body and inhibit the inflammatory response were significantly lower than the expression in the resistant group at seven days postinfection. The expression of Th1 cytokine in the *Hae*III ee-susceptible sheep was significantly lower than that of the *Hae*III dd and *Mva*I cc-resistant groups. However, the expression of IL-4 of Th2 cytokine was significantly increased as compared to that of the *Hae*III dd and *Mva*I cc resistant group. The high expression of IL-2 plays a role against the mycoplasma infection. The interaction of the IL-4 cytokine with eosinophils stimulated the secretion of inflammatory mediators and aggravated the local inflammatory response. These data were consistent with the pathological changes in the sheep infected

with MP in the MO-susceptible/resistance groups. Taken together, it can be concluded that *Hae*III dd and *Mva*I cc were MP-susceptible genotypes, and *Hae*III ee was MO-resistant genotype.

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References

Aarestrup F. M., Jensen N. E. and Østergård H. 1995 Analysis of associations between major histocompatibility complex (BoLA) class I haplotypes and subclinical mastitis of dairy cows. *J. Dairy Sci.* **78**, 1684–1692.

Ammer H., Schwaiger F. W., Kammerbauer C., Gomolka M., Arriens A., Lazary S. *et al.* 1992 Exonic polymorphism vs intronic simple repeat hypervariability in MHC-DRB genes. *Immunogenetics* **35**, 332–340.

Andersson L. and Rask L. 1988 Characterization of the MHC class II region in cattle. The number of DQ genes varies between haplotypes. *Immunogenetics* **27**, 110–120.

Ayling R. D. and Nicholas R. A. J. 2007 Mycoplasma respiratory infections. In *Diseases of sheep*, 4th edition (ed. I. D. Aitken), pp. 231–235. Blackwell Publishing, Oxford, UK.

Ballingall K. T. and Riccardo T. R. 2010 Sequence-based genotyping of the sheep MHC class II DRB1 locus. *Immunogenetics* **62**, 31–39.

Blattman A. W., Hulme D. J., Kinghom B. P., Woolaston R. R., Gray G. D. and Beh K. J. 1993 A search for associations between major histocompatibility complex restriction fragment length polymorphism bands and resistances to Haemonchus contours infection in sheep. *Anim. Genet.* **24**, 277–282.

Bottinelli M., Schnee C., Lepri E., Stefanetti V., Filippini G., Gobbi M. *et al.* 2017 Investigation on mycoplasma populations in pneumonic dairy lamb lungs using a DNA microarray assay. *Small Ruminant Res.* **147**, 96–100.

Briles W. E., Stone H. A. and Cole R. K. 1977 Marek's disease: effects of B histocompatibility alloalleles in resistant and susceptible chicken lines. *Science* **195**, 193–195.

Brown J. H., Jardetzky T. S., Gorga J. C., Stern L. J, Urban R. G., Strominger J. L. *et al.* 1993 Three-dimensional structure of the human class II histocompatibility antigen HLA-DR1. *Nature* **364**, 33–39.

Cheng C., Jun Q., Qingling M., Zhengxiang H., Yu M., Xuepeng C. *et al.* 2015 Serological and molecular survey of sheep infected with *Mycoplasma ovipneumoniae* in Xinjiang, China. *Trop. Anim. Health Pro.* **47**, 1641–1647.

Cimolai N., Taylor G. P., Mah D. and Morrison B. J. 1992 Definition and application of a histopathological scoring scheme for an animal model of acute *Mycoplasma pneumoniae* pulmonary infection. *Microbiol. Immunol.* **36**, 465–478.

Daly A. K. and Day C. P. 2001 Candidate gene case-control association studies: advantages and potential pitfalls. *Brit. J. Clin. Pharmacol.* **52**, 489–499.

Dukkipati V. S. R., Blair H. T., Garrick D. J., Lopez-Villalobos N., Whittington R. J., Reddacliff R. J. *et al.* 2010 Association of microsatellite polymorphisms with immune responses to a killed *Mycobacterium avium* subsp. paratuberculosis vaccine in Merino sheep. *New Zeal. Vet. J.* **58**, 237–245.

Dutia B. M., McConnell I., Ballingall K. T., Keating P. and Hopkins J. 1994 Evidence for the expression of two distinct MHC class II DRB like molecules in the sheep. *Anim. Genet.* **25**, 235–241.

Eiermann T. H., Bettens F., Tiberghien P., Schmitz K., Beurton I., Bresson-Hadni S. *et al.* 1998 HLA and alveolar echinococcosis. *HLA* **52**, 124–129.

Escayg A. P., Hickford J. G., Montgomery G. W., Dodds K. G. and Bullock D. W. 1996 Polymorphism at the ovine major histocompatibility complex class II loci. *Anim. Genet.* **27**, 305–312.

Godot V., Harraga S. and Beurton I. 2000 Resistance/susceptibility to *Echinococcus multilocularis* infection and cytokine profile in humans. II. Influence of the HLA B8, DR3, DQ2 haplotype. *Clin. Exp. Immunol.* **121**, 491–498.

Gorer P. A. 1937 The genetic and antigenic basis of tumour transplantation. *J. Pathol.* **44**, 691–697.

Gottstein B. and Bettens F. 1994 Association between HLA-DR13 and susceptibility to alveolar echinococcosis. *J. Infect. Dis.* **169**, 1416–1423.

Gottstein B. and Hemphill A. 1997 Immunopathology of Echinococcosis. *Chem. Immunol.* **26**, 177–208.

Hediger R., Ansari H. A. and Stranzinger G. F. 1991 Chromosome banding and gene localizations support extensive conservation of chromosome structure between cattle and sheep. *Cytogenet. Genome Res.* **57**, 127–134.

Hemrrann L. M., Brown W. C., Lewic G. S. and Knowes D. P. 2005 Identification and pylogenetic analysis of 15 MHC II DRB1 betal expressed alleles in a ewe-lamb flock. *Immunogenetics* **57**, 855–863.

Hui W. Q., Shen H., Jiang S. and Jia B. 2012 MHC-DQB1 variation and its association with resistance or susceptibility to cystic Echinococcosis in Chinese Merino Sheep. *Asian-Austral. J. Anim. Sci.* **25**, 1660–1666.

Jugo B. M. and Vicario A. 2001 Lymphocyte antigen in sheep: linkage to MHC II DRB1 gene. *Eur. J. Immunogenet.* **28**, 451–458.

Kaufman J. and Venugopal K. 1998 The importance of MHC for Rous sarcoma virus and Marek's disease virus-some Payneful considerations. *Avian Pathol.* **27**, 82–87.

Klein J. 1986 *Natural history of the major histocompatibility complex*, pp. 775. John Wiley, New York.

Konnai S., Nagaoka Y., Takeshima S., Onuma M. and Aida Y. 2003 Sequences and diversity of 17 new Ovar-DRB1 alleles from three breeds of sheep. *Eur. J. Immunogenet.* **30**, 275–282.

La Gruta N., Gras St., Daley S., Thomas P. and Rossjohn J. 2018 Understanding the drivers of MHC restriction of T cell receptors. *Nat. Rev. Immunol.* **18**, 467–478.

Liu C. J., Lee Y. J. and Chang K. W. 2004 Polymorphism of the *MICA* gene and risk for oral submucous fibrosis. *J. Oral. Pathol. Med.* **33**, 1–6.

Li J., Yang Z. Q., Yan G. Q., Li X. P. and Qu Y. G. 2005 Mycoplasma pneumonia epidemiological investigation in Shihezi, Xinjiang. *Pro. Vet. Med.* **26**, 109–110.

Li R.Y., Jia B., Zhang W. J., Zhao Z. S., Shi G. Q., Shen H. *et al.* 2010 Analysis of the relationship between MHC-DRB1 gene polymorphism and hydatidosis in Kazakh sheep. *Asian-Austral. J. Anim. Sci.* **23**, 1145–1151.

Li R. Y., Hui W. Q., Jia B., Shi G. Q., Zhao Z. S., Shen H. *et al.* 2011a The relationship between MHC-DRB1 gene second exon polymorphism and hydatidosis resistance of Chinese Merino (Sinkiang Junken type), Kazakh and Duolang sheep. *Parasite* **18**, 163–169.

Li R.Y., Peng Q. and Jia B. 2011b Antibody and cytokine responses to hydatid in experimentally infected Kazakh Sheep with

hydatidosis resistance haplotype. *Parasitol. Res.* **108**, 1131–1137.

Longenecker B. M. and Gallatin W. M. 1978 Genetic control of resistance to Marek's disease. *IARC Sci. Publ.* **24**, 845–850.

Mahdy E. A., Mäkinen A., Chowdhary B. P., Andersson L. and Gustavsson I. 1989 Chromosomal localization of the ovine major histocompatibility complex (OLA) by in situ hybridization. *Hereditas* **111**, 87–90.

Mejdell C. M., Lie Ø., Solbu H., Amet E. F. and Spooner R. L. 1994 Association of major histocompatibility complex antigens (BoLA-A) with AI bull progeny test results for mastitis, ketosis and fertility in Norwegian Cattle. *Anim. Genet.* **25**, 99–104.

Millot P. 1978 The major histocompatibility complex of sheep (OLA) and two minor loci. *Anim. Blood Group Biochem. Genet.* **9**, 115–121.

Nagaoka Y., Kabeya H., Onuma M., Kasai N., Okada I. C. and Aida Y. 1999 Ovine MHC class II DRB 1 alleles associated with resistance or susceptibility to development of bovine leukemia virus-induced ovine lymphomal. *Cancer Res.* **59**, 975–981.

Nicholas R. A., Ayling R. D. and Stipkovits L. P. 2002 An experimental vaccine for calf pneumonia caused by *Mycoplasma bovis*: clinical, cultural, serological and pathological finding. *Vaccine* **20**, 3569–3575.

Palti Y., Nichols K. M., Waller K. I., Parsons J. E. and Thorgaard G. H. 2001 Association between DNA polymorphisms tightly linked to MHC class II genes and IHN virus resistance in backcrosses of rainbow and cutthroat trout. *Aquaculture* **194**, 283–289.

Paterson S., Wilson K. and Pemberton J. M. 1998 Major histocompatibility complex variation associated with juvenile survival and parasite resistance in a large unmanaged ungulate population (*Ovis aries* L.). *Proc. Natl. Acad. Sci. USA* **95**, 3714–3719.

Renard C., Vaiman M., Chiannilkulchai N., Cattolico L., Robert C. and Chardon P. 2001 Sequence of the Pig major histocompatibility region containing the classical class 1genes. *Immunogenetics* **53**, 490–500.

Rigano R., Bruschi F., Profumo E., Bruschi G. and Carulli G. 2003 Modulation of human immune response by Echinococcus granulosus antigen B and its possible role in evading host defenses. *Infect. Immun.* **89**, 278–286.

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. *Parasitology.* **131**, 403–409.

Schierman L. W. and Collins W. M. 1987 Influence of the major histocompatibility complex on tumor regression and immunity in chickens. *Poult. Sci.* **66**, 812–818.

Schwaiger F. W., Gostomski D., Stear M. J., Duncan J. L., McKellar Q. A., Epplen J. T. *et al.* 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**, 815–822.

Scott P. C., Maddox J. F., Gogolin-Ewens K. J. and Brandon M. R. 1991 The nucleotide sequence and evolution of ovine MHC class II B genes: DQB and DRB. *Immunogenetics* **34**, 80–87.

Sharif S., Mallard B. A., Wilkie B. N., Sargeant J. M., Scott H. M., Dekkers J. C. M. *et al.* 1998 Association of the bovine major histocompatibility complex DRB3 (BOLA-DRB3) alleles with occurrence of disease and milk somatic cell scare in Canadian dairy cattle. *Anim. Genet.* **29**, 85–93.

Shiina T., Inoko H. and Kulski J. K. 2004 An update of the HLA genomic region, locus information and disease associations. *HLA* **64**, 631–649.

Shen H., Han G., Jia B., Jiang S. and Du Y. 2014 *MHC-DRB*1/*DQB*1 Gene polymorphism and its association with resistance/susceptibility to cystic Echinococcosis in Chinese Merino sheep. *J. Parasitol. Res.* **2014**, 1–6.

Song Q. Y., Zhang Y. J., Liu Y. Q. and Li T. Q. 2011 Antimicrobial activities of eighteen antimicrobials against *Mycoplasma ovipneumoniae* and *Mycoplasma mycoides* isolates in vitro. *Pro. Vet. Med.* **32**, 14–18.

Tao Y., Li X. P., Yan G. Q. and Li W. M. 2006 Epidemiological survey of sheep contagious pleuropneumonia and the preliminary research on its prevention and control in Shihezi, Xinjiang. *Chinese J. Prevention Vet. Med.* **33**, 62–64.

Tao Y., Li X. P., Liu G., Zhang X. E., Lin W. M. and Zheng Z. H. 2007 Separation of Mycoplasma pneumonias pathogen and its regression test. *China Anim. Husbandry Vet. Med.* **34**, 112–113.

Tissot R. G., Beattie C. W. and Amoss M. S. 1989 Association of the SLA complex with Sinclair swine cutaneous malignant melanoma. *Anim. Genet.* **20**, 51–57.

Torcal M., Lozano L. and Larrad J. C. 1996 Immune response and in vivo production of cytokines in patients with liver hydatidosis. *Clin. Exp. Immunol.* **106**, 317–322.

Turner M. W. 1996 Mannose-binding lectin: the pluripotent molecule of the innate immune system. *Immunol. Today* **17**, 532–540.

Trier N., Izarzugaza J., Chailyan A., Marcatili P. and Houen G. 2018 Human MHC-II with shared epitope motifs are optimal Epstein-Barr virus glycoprotein 42 ligands—relation to rheumatoid arthritis. *Int. J. Mol. Sci.* **19**, 317.

Ujvari B. and Belov K. 2011 Major histocompatibility complex (MHC) markers in conservation biology. *Int. J. Mol. Sci.* **12**, 5168–5186.

Zhang W., Li J. and McManus D. P. 2003 Concepts in immunology and diagnosis of hydatid disease. *Clin. Microbiol. Rev.* **16**, 18–36.

Zhang H., Zhao Z. S., Zhao F. and Yu P. 2012 The relationship between MBL genotype and lung histopathologic lesions of *Mycoplasma ovipneumoniae* pneumonia in sheep. *Chin. J. Prevent. Vet. Med.* **34**, 293–296.

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