ASSOCIATIONS BETWEEN INFECTIONS WITH *HAEMONCHUS CONTORTUS* AND GENETIC MARKERS ON OVINE CHROMOSOME 20

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INTRODUCTION

Evaluation of different species and breeds in different breeding situations and environments have demonstrated the possibility of breeding for nematode resistance (Gray, 1997; Gauly *et al.*, 2001; Gauly and Erhardt, 2001). The identification of genetically resistant animals could be facilitated if the genomic loci responsible for the majority of the genetic variation in host resistance would be known. In addition, the identification of genes which regulate resistance would improve our understanding of the mechanisms of host defence against parasites and could accelerate the development of efficient vaccines. Hints for genetic resistance in sheep against *Haemonchus contortus* are given by studies of Bouix *et al.* (1998) and Outteridge *et al.* (1996) for example. Associations in Soay sheep between resistance to intestinal nematodes and OLADRB alleles which is a genetic marker belonging to the *Major Histocompatibility Complex* (MHC) were demonstrated (Paterson *et al.*, 1998). The MHC is located to ovine chromosome 20 (De Gortari *et al.*, 1998). Further on associations between MHC and eggs per gram of faeces (EpG) were demonstrated for *Ostertagia circumcincta* (Schwaiger *et al.,* 1995) and *Trichostrongylus colubriformis* (Luffau *et al.*, 1986). However associations between genetic markers and *Haemonchus contortus* infections have so far not been estimated. The aim of this study was to calculate associations between parameters of parasite resistance and genetic markers on OAR 20.

MATERIAL AND METHODS

Animals. Five parental half sib families of the German breed Rhönschaf (n = 468) kept at the Research Station Oberer Hardthof of the Department of Animal Breeding and Genetics of the University in Giessen were used in this study.

Infection and sample collection. Lambs were weaned and infected orally with 5000 *Haemonchus contortus* L_3 larves at an age of 12 weeks. Four and eight weeks *post infectionem (p.i.)* faeces and blood samples were taken from all lambs. Eggs per gram of faeces (EpG), haematocrit level and serum IgL level were evaluated .

Microsatellite typing. Six microsatellites from ovine *OAR 20* in a distance of 15-20 cM were used for genotyping (Crawford *et al.,* 1995; De Gortari *et al.,* 1998; Buitkamp *et al.*, 1996): *OarCp73*, *BM1815*, *ODRBps*, *OarHH56*, *BM1905* and *DYMS1*. DNA was isolated from leucocytes according to Montgomery and Sise (1990). PCR was performed in 15µl volume using 50 ng of genomic DNA, 10 pM of each primer, 200 µM of each dNTP, 0.4 U Taq polymerase (Hybaid) and PCR buffer (20 mM Tris-HCl, pH 8.55; 1.6 mM NH_4SO_4). Amplification was done in a Perkin Elmer Gene Amp PCR system 9600 cycler. The forward primer of each marker was Cy5 end-labelled (Amersham Pharmacia, Freiburg). Genotyping was done using an automated laser detection system (A.L.F. *express*, Amersham Pharmacia

Freiburg). PCR-products were run on a 5.5% long ranger gel (0.5 mm, 6 M urea) and analysed with the program AlleleLinks v. 1.00. (Amersham Pharmacia, Freiburg).

Statistical analyses. All statistical analyses were done using the software package SPSS (version 9.0). To produce normally distributed data the EpG was transformed with the decimal logarithm (LogEpG). Heterozygosity for each marker was estimated according to Botstein *et al.* (1980). For association studies the allele with the highest frequency was postulated as population standard for each marker. A regression analysis within a linear model with effect of sex (fix), effect of the ram (random) and weight (co variable) was used to test the effect of allele substitution. Only alleles with a frequency higher than 5% were used in this analyses. According to the number of tested alleles (n) a Bonferroni correction was done (Sokal and Rohlf, 1995) by adjusting the significance level to p=0.05/n.

RESULTS AND DISCUSSION

Parameters of parasite resistance. Table 1 shows the least square means and standard errors of the parameters of parasite resistance four and eight weeks *post infection*. At the second sampling date logEpG and the *Haemonchus contortus* specific IgL level increased in comparison to the first sampling date.

Table 1. Least-square (LSQ) means and standard errors (s.e.) of the parameters of parasite resistance

Parameter	4 weeks *p.i.*		8 weeks *p.i.*	
	LSQ-Mean	s. e.	LSQ-Mean	s. e.
LogEpG	3.32	0.231	3.46	*0.359*
Haematocrit	*0.23*	*0.031*	0.24	0.036
IgL	1.64	0.450	2.62	0.461

Number of alleles. The number of identified alleles and the number of alleles used in the association study is shown in table 2. New significance level after the Bonferroni correction are also pointed out. From the 9 alleles of marker *BM1815* identified in Rhönschaf, only four had a frequency higher than 5%. The heterozygozity of the microsatellites were between 0.159 and 0.794 respectively.

Table 2. Number of alleles and Bonferroni correction of the significance level

Marker	Number of identified alleles	Number of alleles used	Significance level (Bonferroni correction)
OarCP73	4	3	0.0250
DYMS1	6	5	0.0125
BM1815	9	4	0.0167
ODRBps	8	5	0.0125
OarHH56	5	3	0.0250
BM1905	2	2	0.0500

Association studies. Significant associations between alleles of different microsatellites and parameters of parasite resistance are shown in table 3. Associations were found between the marker OarCP73 and the haematocrit level at both sampling dates. This marker is closely linked to the erythrocyte antigen C. The allele A of the microsatellite *OarCP73* had a positive effect on haematocrit level of 6.78% at the first sampling date and an effect of 9.20 % at the second sampling date. On the other hand the allele B of the same marker had a negative effect of –18.74 % on the haematocrit level 8 weeks p.i. (sample2). A further association was found between the marker DYMS 1 and the IgL Level. The marker DYMS1 was developed from the DYA gene in sheep which belongs to the class IIb subregion of the MHC (Buitkamp *et al.*, 1996). Allele C of this marker reduces the IgL-Level by 15.60 % in the second sample. Another association was found between the allele C of marker *BM1815* and the LogEpG 4 weeks p.i. (sample 1) Substitution of the standard allele by the allele C reduced the LogEpG by 5.56 %.

Table 3. Significant effects of alleles on parameters of parasite resistance compared to the standard allele of the marker

Marker	Allele	Parameter	Sample[1]	Significance	Effect	Effect in %
OarCP73	A	Haematocrit level	1	0.025	$1.956*10^{-2}$	6.78
OarCP73	A	Haematocrit level	2	0.022	$2.181*10^{-2}$	9.20
OarCP73	B	Haematocrit level	2	0.002	$-4.443*10^{-2}$	-18.74
DYMS1	C	IgL-Level	2	0.007	-0.409	-15.60
BM1815	C	LogEpG	1	0.015	-0.205	-6.18

[1] 1: 4 weeks *p.i.* 2: 8 weeks *p.i.*

Previous studies of Buitkamp *et al.* (1996) found an association between the microsatellite DYMS1 and faecal egg counts of *Ostertagia circumcincta* and Schwaiger *et al.* (1995) identified associations between alleles of the DRB1 locus and faecal egg counts of *Ostertagia circumcincta* in similar dimensions. Our results demonstrate the significant role of MHC for parasite resistance traits also in Rhönsheep.

CONCLUSION

Significant effects were found between parameters of parasite resistance and the markers *OarCP73*, *DYMS1* and *BM1815*. The DYA gene which is closely linked to the microsatellite DYMS1 may be a possible candidate gene for resistance against *Haemonchus contortus* in sheep.

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