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Genetic parameters for resistance to trichostrongylid infection in dairy sheep

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In sheep, the traditional chemical control of gastrointestinal nematode (GIN) parasites with anthelmintics has led to the widespread development of anthelmintic resistance. The selection of sheep with enhanced resistance to GIN parasites has been suggested as an alternative strategy to develop sustainable control of parasite infections. Most of the estimations of the genetic parameters for sheep resistance to GIN parasites have been obtained from young animals belonging to meat- and/or wool-specialised breeds. We present here the estimated genetic parameters for four parasite resistance traits studied in a commercial population of adult Spanish Churra dairy ewes. These involved two faecal egg counts (FECs) ($LFEC_0$ *and* $LFEC_1$*) and two serum indicator traits, the anti-*Teladorsagia circumcincta *fourth stage larvae IgA (*IgA*) and the pepsinogen (*Peps*) levels. In addition, this study has allowed us to identify the environmental factors influencing parasite resistance in naturally infected Spanish Churra sheep and to quantify the genetic component of this complex phenotype. The heritabilities estimated for the two FECs analysed (0.12 for* $LFEC_0$ *and 0.09 for* $LFEC_1$*) were lower than those obtained for the examined serum indicators (0.19 for* IgA *and 0.21 for* Peps*). The genetic correlations between the traits ranged from 0.43 (*Peps−IgA*) to 0.82 (*$LFEC_0$−$LFEC_1$*) and were higher than their phenotypic counterparts, which ranged between 0.07 and 0.10. The heritabilities estimated for the studied traits were lower than previously reported in lambs. This may be due to the differences in the immune mechanisms controlling the infection in young (antibody reactions) and adult (hypersensitivity reactions) animals/sheep. In summary, this study demonstrates the presence of heritable variation in parasite resistance indicator traits in the Churra population studied, which suggests that genetic improvement is feasible for this complex trait in this population. However, further studies in which the experimental variables are controlled as much as possible are needed to identify the best trait that could be measured routinely in adult sheep as an indicator of parasite resistance.*

Keywords: gastrointestinal parasites, genetic resistance, heritability, dairy sheep

Implications

This study addresses the estimation of genetic parameters of four indicator traits of parasite resistance in a commercial population of dairy sheep. The phenotypes studied, which included two faecal egg counts (*$LFEC_0$* and *$LFEC_1$*) and two serum indicator traits, the anti-*Teladorsagia circumcincta* fourth stage larvae IgA and the pepsinogen levels, were measured in adult ewes. This study represents the first step to implement genetic selection for parasite resistance in the Churra breed. The results presented herein could have very important implications through the reduction in the use of anthelmintic treatments and the production of safer sheep products.

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Introduction

Internal parasite infections pose a major health problem to domestic livestock worldwide (Perry *et al.*, 2002), particularly in those animals reared in the grazing livestock systems. In sheep, gastrointestinal nematode (GIN) parasites cause important economic losses. The direct losses are because of the control of the infection, veterinary care and the death of the severely affected animals. Additionally, there are indirect losses due to a reduction in performance

(Nieuwhof and Bishop, 2005), which is in many cases caused by the subclinical effects of the infections. Several studies have shown that subclinical diseases can result in a reduction in live weight (Coop *et al*., 1985; Mackay *et al*., 1998), wool weight (Donald, 1979) and milk production (Leyva *et al*., 1982; Thomas and Ali, 1983).

Traditional chemical control of GIN parasites with anthelmintics has led to the widespread development of anthelmintic resistance by most of the prevalent nematode parasites of small ruminants (Waller, 1997). Furthermore, the possible presence of chemical residues in the products derived from animals treated with anthelmintics is an important issue from a public health point of view. Therefore, strategies to develop the sustainable control of GIN parasites should be implemented now, which in the long term could lead to a reduction in the use of anthelmintic compounds. Among the proposed alternative strategies, the selection of sheep with enhanced resistance to GIN parasites has been suggested by many authors (Woolaston and Baker, 1996; Gray, 1997; Sayers and Sweeney, 2005). This was based on evidence acquired over the last several decades demonstrating that a significant proportion of the variation in sheep resistance to internal parasites is genetically determined (reviewed by Raadsma *et al*., 1997). The number of parasite eggs in the faeces, the faecal egg count (FEC), is the most widely used as an indicator trait regarding GIN parasite resistance. The heritability estimates reported in the literature for the FEC show a wide range of variation, from 0.0 to 0.55, as reviewed by Raadsma *et al*. (1997) depending on different factors, such as the population studied (e.g. sheep breed or animal age) or the nature of the parasite challenge (e.g. natural or artificial infection). Most of the estimations of genetic parameters for sheep resistance to GIN parasites have been carried out in sheep populations specialised for meat and/or wool production and managed under grazing-extensive production systems (Bishop *et al*., 2004; Morris *et al*., 2004), and the animals studied in most of the cases were lambs.

In this study, we studied indicators of parasite resistance in a commercial population of the Spanish Churra sheep breed, an autochthonous dairy breed from the region of Castilla y León, where the traditional breeding system is based on indigenous grazing breeds. The Churra breed is one of the most adapted breeds to the prevailing climatic conditions of the region, grazing most of the year. Despite the low worm burden of pastures during the dry season, Churra breeders use anthelmintic treatment to control infections. However, *Strongylid* nematode parasites are known to cause substantial production losses in Churra sheep flocks because of a subclinical infection and a reduction of general immune responses even when the infections are mild (Rojo-Vázquez, 2004). In addition, the infection of young females turned out to pasture for the first time may lead to clinical signs of disease, such as diarrhoea and even death (Rojo-Vázquez, 2004). With the aim of minimising the use of anthelmintics in the future that are required to control parasite infections in Churra sheep, this work examined the study of the genetic control of GIN parasite infection in this sheep breed. Using a commercial population of Churra sheep flocks exposed to natural infection, we identified the environmental factors that have a significant influence on parasite resistance traits and estimated the proportion of the variation of GIN parasite resistance that is under genetic control in this breed.

Material and methods

Sampled animals and measurements

The sampling period began in October 1999 and lasted until September 2003; 1545 records from 928 ewes, which belonged to 104 half-sib families, were collected from 5 different flocks. The average number of daughters per ram was 8.92, ranging between 3 and 127. The flocks were genetically connected through the use the artificial insemination (AI). Hence, 27 out of the 104 sires included in the study belonged to the AI program established by the National Association of Spanish Churra Sheep Breeders and contributed to the study with a high number of daughters. Fourteen of these sires had offspring in the five studied flocks. Three generations were used when calculating the genetic parameters.

At the beginning of the experiment (F_0 day), the animals were de-wormed and faecal samples were taken to obtain the initial faecal egg count (FEC_0). On the basis of information from the farmers, the possible existence of anthelmintic resistance and the degree of basal infection were formally assessed for each flock. Therefore, the de-worming protocol (type of drug and dosage) was specifically designed for each flock. Approximately 2 months after this day (F_{60} day), faecal and blood samples were collected from the same animals. These samples were processed to obtain the FEC at day 60 (FEC_1), the serum IgA (*IgA*) and the blood pepsinogen (*Peps*) levels.

Owing to management, lambing dates and weather conditions, the interval-sampling period (ID_0-D_{+60}) showed some deviation from the originally planned 60 days (range 42 to 124 days, mean 73 days; s.d. = 22.3). During the 2 months of the experiment, the animals were managed under standard practices in commercial Churra dairy sheep populations, which included daily grazing. Therefore, the animals were exposed to natural parasite infections during the course of the study.

Faecal analysis. The number of eggs per gram – (epg) of faeces is a measure of eggs produced by female parasites within the host animal and is thought to be a good indicator of the parasite infection status of the host (Stear *et al*., 1995). A modified McMaster technique using zinc sulphate (d = 1.33) was used to determine the number of nematode epg with a sensitivity of 15 epg. Bulked faecal samples were taken and cultured for estimation and identification of the nematode genera present following the Ministry of Agriculture, Food and Fisheries (MAFF) identification keys (Ministry of Agriculture, Food and Fisheries,

1986). The minimum number of sampled animals per flock was 30. Eggs from different parasite groups were identified as *Strongylid* nematode parasites, *Nematodirus* spp., *Moniezia* spp., *Trichuris* spp., *Dicrocoelium dentriticum*, *Fasciola hepática*, and oocysts from *Eimeria* spp. A new variable, the total FEC, was generated as follows:

$$\text{FEC} = \text{strongyles FEC} + (\textit{Nematodirus}\ \text{FEC} \times 10).$$

This formula takes into account the lower prolificacy of the *Nematodirus* genera (5%) when compared with other strongyles such as *Teladorsagia* (55%), based on the observations reported by Kates (1947).

Bulk faecal cultures for each sampling were carried out. Third stage larvae (LIII) were identified by morphological characteristics following the keys from MAFF (Stear and Bishop, 1999). The *Teladorsagia* LIII percentage in the coproculture was registered as a possible factor in the model (TEL).

Serum analysis. Blood samples were obtained by venipuncture in the jugular vein. Serum was stored at −80°C until processing. An immunological trait indicator, the serum anti-*Teladorsagia circumcincta* LIV IgA level (*IgA*), was determined in an ELISA based on the technique described by Martínez-Valladares *et al*. (2005a).

The serum *Peps* level, which is an indicator of gastric damage associated with the progression of larvae to the adult stage (Murray *et al*., 1970), was measured by fluorometric determination in a 96-well microtitre plate using the adapted technique from Edwards *et al*. (1960).

Data analysis

Before further analysis, the distributions of the data were assessed. The distribution values for FEC_0 and FEC_1 were positively skewed and were transformed using a logarithm transformation (L $FEC_0 = \ln(FEC_0 + 1)$; L $FEC_1 = \ln(FEC_1 + 1)$), which resulted in more symmetrical distributions for these traits. The *IgA* and *Peps* data did not require any transformation.

To assess the variables influencing the parasite resistance-related traits, the following mixed model was fitted to the data by means of an analysis of variance (ANOVA) performed through the MIXED procedure using the Statistic Analysis System programme, SAS package (SAS User's Guide, 1998). Data were fitted to the following general model:

$$Y_{ijklmn} = \mu + F_i + M_j + LN_k + PhS_l + Sire_m + e_{ijkmn},$$

where Y_{ijklmn} is a dependent variable measured for each of the analysed traits (FEC_0, FEC_1, *IgA* and *Peps*), μ represents the population mean, F_i is the fixed effect of the flock i (five levels), M_j represents effect of sampling month j (eight levels), LN_k is the fixed effect of the lambing number k (six levels), PhS_l represents the fixed effect of the physiological status based on the number of days between parturition and sampling (four levels: pregnant, peripartum, dairy and dry-non pregnant), $Sire_m$ is the random effect of the sire m, and e_{ijklmn} is the residual effect. In addition, the number of days between the first and the second sampling (sampling interval, ID_0-D_{+60}) was considered as a covariate for $LFEC_1$.

Phenotypic correlations were calculated with the CORR procedure of the SAS package (SAS User's Guide, 1998). Genetic parameters and their standard errors were estimated using the restricted maximum likelihood estimation on the basis of analytical gradients (AG-REML) procedure (Neumaier and Groeneveld, 1998) and the VCE package rel. 4.2 (Groeneveld, 1998). In the univariate analysis of each trait, data were gathered according to the different levels of the main environmental variables demonstrating a significant influence ($P < 0.05$) on the parasite resistance-related trait under study in the initial ANOVA. The influence of the flock and month fixed factors was taken into account as the flock-year-season of the sampling factor, which had 17 levels. Each year was divided into three conventional seasons, January to March, April to June and July to December, according to previous studies in Churra sheep (de la Fuente *et al*., 1995). All known pedigree relationships between individuals were considered in the model. Data of the four dependent variables under study, FEC_0, FEC_1, *IgA* and *Peps*, were analysed with the following multitrait repeatability animal model:

$$Y_{ijkl} = \mu + FYS_i + LN_j + A_k + PE_k + e_{ijkl},$$

where Y_{ijkl} is the dependent variable, μ is the population mean, FYS_i is the fixed effect of flock-year-season i, LN_j is the fixed effect of lambing number j, A_k is the additive genetic random effect of the individual k, PE_k is the permanent environmental random effect on the individual k and e_{ijkl} is the random residual effect. For $LFEC_1$, the sampling interval ID_0-D_{+60} was also considered in the calculation of genetic parameters as a covariate. It is important to note that the effect of the physiological status, *PhS*, caused a large distortion when it was introduced in the model fitted with VCE. For this reason this factor was not included in the animal model used for the calculation of the genetic parameters.

Results

Summary statistics

The mean, maximum and minimum values as well as the standard deviation for all the traits investigated are shown in Table 1. Note that in order to simplify the interpretation of data, the basic statistics shown for $LFEC_0$ and $LFEC_1$ are based on the raw untransformed data. These data refer to total egg counts in which the most prevalent genera encountered was *Teladorsagia* (65.5%), followed by *Trichostrongylus* spp. (30.5%), *Nematodirus* spp. (3.1%) and some less frequent genera (1% *Chabertia* spp. and *Oesophagostomum* spp.). The initial egg count, FEC_0, showed

Table 1 *Descriptive statistics of phenotypes related to parasite resistance analysed in this study*

Trait	Total number of observations (number of ewes)	Mean	Minimum	Maximum	% 0 values	s.d.
FEC_0 (eggs/g faeces)	1513 (928)	260.01	0	6062	12.89	237.01
FEC_1 (eggs/g faeces)	1301 (928)	104.11	0	3327	31.82	134.50
Peps (mUTyr)	1175 (928)	231.92	0	1583	1.96	199.53
IgA (OD ratio)	1160 (928)	0.23	−0.56	2.32	1.64	0.28

Peps = pepsinogen; OD = optical density.
The total number of observations analysed, mean, range, percentage of 0 values and s.d. are given for each studied trait.
Note: FEC_0 and FEC_1 are presented as untransformed values and refer to the total number of eggs (all genera of parasites).

Table 2 *Statistical significance results from the variance analysis performed for the four parasite resistance traits analysed in this study*

Factors considered in the model	Source of Variation	Trait			
		$LFEC_0$	$LFEC_1$	*Peps*	*IgA*
Fixed factors	Flock	25.42***	19.24***	10.61***	11.39***
	Month	23.99***	10.14**	12.89***	7.55***
	Lambing Number	2.46*	4.19***	1.17 ns	1.12 ns
	Physiological Status	2.28*	22.06***	4.01*	2.39 ns

Peps = pepsinogen; ns = non-significant.
Statistical significance of *F*-values: ***$P < 0.001$; **$P < 0.01$; *$P < 0.05$; ns $P > 0.05$.
The statistical significance (*F*-value) of the fixed effects included in the model are given for all variables studied.

Table 3 *Least square means obtained for the fixed effects included in the model of the variance component analysis performed for the parasite resistance traits examined in this study*

	Level	$LFEC_0$	$LFEC_1$	*Peps*	*IgA*
Flock	A	2.89 ± 0.25	2.71 ± 0.28	226.83 ± 19.05	0.20 ± 0.06
	B	5.09 ± 0.33	3.11 ± 0.38	345.58 ± 41.67	0.01 ± 0.07
	C	4.53 ± 0.20	3.92 ± 0.22	262.26 ± 16.85	0.37 ± 0.06
	D	6.67 ± 0.47	2.82 ± 0.53	345.50 ± 47.91	0.30 ± 0.12
	E	3.82 ± 0.26	0.57 ± 0.30	338.32 ± 21.68	0.23 ± 0.07
Lambing number	1	4.30 ± 0.17	2.91 ± 0.19	316.71 ± 17.63	0.18 ± 0.03
	2	4.82 ± 0.18	2.64 ± 0.20	312.90 ± 18.37	0.23 ± 0.03
	3	4.53 ± 0.19	2.38 ± 0.22	279.49 ± 19.48	0.20 ± 0.03
	4	4.60 ± 0.19	2.40 ± 0.22	294.41 ± 19.28	0.20 ± 0.03
	5	4.52 ± 0.20	2.31 ± 0.23	317.17 ± 19.39	0.24 ± 0.04
	6>	4.83 ± 0.17	3.11 ± 0.19	301.02 ± 17.59	0.25 ± 0.03
Physiological Status	Pregnant	4.83 ± 0.16	2.91 ± 0.18	310.05 ± 18.85	0.20 ± 0.04
	Dairy	3.31 ± 0.16	1.99 ± 0.18	290.13 ± 16.40	0.14 ± 0.04
	Dry-non pregnant	4.55 ± 0.22	2.18 ± 0.25	272.47 ± 21.57	0.18 ± 0.04
	Peripartum	4.70 ± 0.16	3.42 ± 0.18	332.20 ± 17.03	0.19 ± 0.04

Peps = pepsinogen.

higher faecal counts than $LFEC_1$, with mean values of 260.01 and 104.11 epg, respectively.

Influence of environmental factors

The results of the ANOVA are summarised in Table 2. Least square means calculated for three of the fixed factors included in the ANOVA analysis of the different traits (flock, physiological status and lambing number), are provided in Table 3. $LFEC_0$ was mainly affected by month ($P < 0.001$) and flock ($P < 0.001$), with a lower influence of the lambing number ($P < 0.05$) and the physiological status of the ewe ($P < 0.05$).

$LFEC_1$ was also affected by all the factors considered in the general model. The sampling interval (ID_0-D+_{60}), which was only included in the analysis of $LFEC_1$, contributed significantly ($P < 0.001$) to the variation of this trait.

Peps and *IgA* were found to be significantly affected ($P < 0.001$) by flock and month, with the physiological status showing only a moderate influence on the *Peps* level ($P < 0.05$).

Lambing number had a significant influence on both the FECs but did not influence the *Peps* and *IgA* levels. The highest $LFEC_0$ and $LFEC_1$ were found in the older ewes (lambing number $\geqslant 6$) (Table 3). $LFEC_1$ appeared to be the most influenced trait by the ewe's physiological status, with the animals at the peripartum period showing the highest FEC for this trait. This physiological status showed also the highest *Peps* level, although the influence on this trait, and also on $LFEC_0$, was less significant ($P < 0.05$).

Phenotypic and genetic parameters

The heritabilities and proportions of permanent environmental variance (c^2) estimated for the parasite resistance traits examined with the AG-REML analysis are given in the Table 4 (diagonal). The estimated heritabilities ranged from 0.09 to 0.21. The serum traits *IgA* and *Peps* had higher heritability estimates than the FEC traits. The heritabilities for $LFEC_0$ and $LFEC_1$ were low-to-moderate, displaying slightly higher values for $LFEC_0$ than for $LFEC_1$ (0.12 and 0.09, respectively). This might be explained by the higher percentage of 0 values for $LFEC_1$ (31.8%) compared to those obtained for $LFEC_0$ (12.9%). *Peps* and *IgA* appeared to be moderately heritable, with estimates of 0.21 and 0.19, respectively. The proportions of permanent environmental variance calculated ranged from 0.02 (for $LFEC_1$) to 0.27 (for $LFEC_0$). For the serum traits this parameter showed values of 0.04 for *Peps* and 0.09 for *IgA.*

The genetic and phenotypic correlations between the four parasite resistance traits are also provided in Table 4. The correlations that were significantly different from 0, according to their standard errors, are shown in bold. Both phenotypic and genetic correlations between $LFEC_0$ and *Peps* and $LFEC_1$ and *IgA* were not found to be significantly different from 0.

Considering the significance estimates, phenotypic correlations appeared to be weak, with values in the range of 0.07 to 0.10. Three out of the four significant phenotypic correlations showed positive signs, with only a negative environmental correlation, between $LFEC_1$ and *Peps* (-0.11).

For all the cases, the genetic correlations were higher than the phenotypic counterparts, although they showed similar patterns and signs to those observed for the phenotypic traits. The significant estimates for the genetic correlations ranged from 0.43 between *Peps* and *IgA* to 0.82 between $LFEC_0$ and $LFEC_1$. A high negative genetic correlation was found between *Peps* and $LFEC_1$ (-0.51), whereas *IgA* showed positive and moderately high genetic correlations with $LFEC_0$ and *Peps* (0.45 and 0.43, respectively).

Discussion

In this study, we identified the environmental factors influencing parasite resistance in a commercial population of adult Churra sheep under natural infection and quantified the genetic component of this complex trait. As indicators of parasite resistance, we measured FECs, the plasma IgA concentration and the serum *Peps* level. These traits are likely to represent different aspects related to the host-parasite interactions during infection.

The phenotypic traits measured in this study are influenced by both environmental and genetic factors. The flock and sampling month were the external factors significantly influencing all the studied traits. This reflects the importance of the management given provided to the sheep, including management practices and protein content level in the diet (Martínez-Valladares *et al.*, 2005b), and the influence of the season as a factor directly controlling parasite development, especially in continental climates where hypobiosis phenomena are frequent (Almería *et al.*, 1996).

Other factors, such as age and the physiological status of the ewe, significantly influenced several of the traits studied. In our model, the age of the ewe is considered within the lambing number factor, which also takes into account the productivity stress accumulated during the animal's lifetime. Our analysis indicated that older ewes had a tendency to have higher FECs (the highest for $LFEC_1$ and the second highest one for $LFEC_0$) indicating that, even without suffering obvious production loses, the animals may show a hypersensitivity reaction to the incoming larvae. The lack of a significant influence of lambing number on the IgA level suggests that the host's ability to recognise parasite-specific antigens and develop an IgA response to control the infection appeared to be independent of the age of the animal. This observation seems reasonable considering that

Table 4 *Genetic and phenotypic parameters calculated for the parasite resistance traits examined in this study*

	$LFEC_0$	$LFEC_1$	*Peps*	*IgA*
$LFEC_0$	0.12 ± 0.04 (*0.27 ± 0.05*)	**0.82 ± 0.11**	-0.19 ± 0.11	**0.45 ± 0.20**
$LFEC_1$	**0.10 ± 0.03**	0.09 ± 0.03 (*0.02 ± 0.03*)	**−0.51 ± 0.15**	0.09 ± 0.25
Peps	0.03 ± 0.03	**−0.10 ± 0.03**	0.21 ± 0.04 (*0.04 ± 0.03*)	**0.43 ± 0.19**
IgA	**0.07 ± 0.03**	0.04 ± 0.03	**0.08 ± 0.01**	0.19 ± 0.05 (*0.09 ± 0.05*)

Peps = pepsinogen.

Heritabilities (on the diagonal) and genotypic (above the diagonal) and phenotypic (below the diagonal) correlations between the parasite resistance traits studied in the present work. The proportions of permanent environmental variance (c^2) are also given in the diagonal (in italics and between brackets). The correlations found to be significantly different from 0, according their standard errors, are shown in bold.

we only analysed adult ewes; therefore, all the animals became immunocompetent several months before the initiation of this experiment. According to Stear *et al.* (1999c), the immunity of sheep against *T. circumcincta* develops in two stages. Young animals control worm growth and fecundity by producing parasite-specific antibodies. Subsequently, as the animals get older, they control worm numbers through the acquisition of effective immune responses that reduce the number of worms, possibly through immediate hypersensitivity reactions against incoming third-stage larvae (Stear *et al.*, 1999c). Also the *Peps* levels appeared to be independent of the animal's age.

In accordance with other studies, we have observed higher FECs in the periparturient ($LFEC_1$) and pregnant ewes ($LFEC_0$), which has been suggested to be the result of a periparturient breakdown of immunity because of prioritised nutrient allocation to reproductive functions rather than to immune functions (Houdijk *et al.*, 2003) and a failure of pregnant ewes to expel the resulting adult worms (Gibbs and Barger, 1986). Jeffcoate *et al.* (1990) observed an increase in the plasma IgA level in periparturient ewes when IgA is transported from the gut to milk during early lactation, leading to a temporary reduction in the abomasal antibody level of ewes. This would permit the establishment and development of inhibited larvae, leading to a periparturient rise in the FEC. However, we did not observe a significant effect of the physiological status of the ewe on the serum *Peps* and *IgA* levels.

As expected, the FEC measured on day 60 of the experiment ($LFEC_1$) was influenced by the sampling interval (ID_0-D_{+60}) and showed significantly positive correlation (phenotypic and genetic) with the initial egg count ($LFEC_0$), which is an indicator of pasture contamination before $LFEC_1$ sampling. It should be taken into account that at the beginning of the experiment, when FEC_0 was measured, the experimental conditions (e.g. last anthelmintic treatment and correct administration of drugs) were not totally controlled. This may explain some of the differences regarding the significance level of the effects of some of the factors considered in the model, for example, the larger significance of the lambing number and the physiological status on the second egg count than on the initial egg count. The lack of control of the experimental conditions at the time of measuring $LFEC_0$ is also evidenced by the much higher proportion of phenotypic variance because of the permanent environment observed for this trait than that estimated for the traits measured after the sampling interval (ID_0-D_{+60}) (Table 4). As expected, the anthelmintic treatment had a drastic effect on reducing the influence of the animal's permanent environment on the parasite resistance traits measured at D_{+60}.

Regarding the heritabilities estimated in this study, both FECs showed similar but lower values than those generally reported in lambs (Windon, 1996; Stear *et al.*, 1997; Morris *et al.*, 2004; Davies *et al.*, 2006). Genetic parameters for parasite resistance in adult sheep are poorly described. Interesting results have been reported by Beraldi *et al.* (2007) in a free-living Soay sheep population in which no genetic variation for strongyle FECs and a very low heritability for coccidian faecal oocyst count were detected in adult sheep. The same authors reported moderate heritability estimates for the same traits measured in lambs (0.22 to 0.26), which are in the range of other authors' estimates for the same trait in young animals. The lower heritabilities estimated for the FECs in adult Churra and Soay sheep when compared to lambs may be due to the differences in the immune mechanisms controlling the infection in young and adult animals, as we previously commented. Therefore, it is possible that the control of worm number by adult sheep, mediated through immediate hypersensitivity reactions, has genetic components that are not well represented by the classic indicator traits used to measure resistance in lambs, in which the major manifestation of resistance is the control of worm growth and worm fecundity through the production of antibodies (Stear *et al.*, 1999b). In this regard, it seems interesting that the heritability estimates of FECs in lambs reported by Davies *et al.* (2006) decreased slightly as animals grew, with heritability estimates for FEC at 4, 5 and 6 months, being 0.30, 0.21 and 0.19, respectively.

The relatively short interval between the initial anthelmintic treatment and the measurement of $LFEC_1$ may not have been enough to guarantee an equal infection level in all the animals. This fact might explain the slightly higher heritability estimated for $LFEC_0$ (0.12) *v.* that estimated for $LFEC_1$ (0.09). As the animals were subjected to natural uncontrolled exposure to parasite infection after the initial anthelmintic treatment, it is likely that at the time of the $LFEC_1$ measurement, several ewes had not been infected at all. Furthermore, for those that had been infected during the sampling interval, the parasites would still be in a pre-adult stage. Taking this into account, we believe that $LFEC_0$ could be considered an indicator of general parasite resistance in this population, even when the experimental conditions were not completely controlled at the beginning of the experiment, whereas the traits measured on D_{+60} are likely to be better indicators of initial immune responses triggered by larvae at the beginning of the infection.

The serum traits (*Peps* and *IgA*) showed higher heritability than the FECs. The heritability of *IgA* reached a medium value, which is consistent with that reported in Scottish blackface lambs by Davies *et al.* (2006). The highest heritability was that estimated for *Peps* (0.21), although it was markedly lower than that estimated by Davies (2004) for the same trait in Scottish blackface lambs (0.56). The higher heritability estimated for *Peps* and *IgA* compared to the FECs should be considered when selecting traits to be included in a breeding program. However, these results should be considered cautiously because of the limited control of the experimental conditions at the beginning of the study and the relatively short sampling interval between both FECs.

This study has allowed us to quantify the relationships between the parasitic, immunological and biopathological

indicator traits studied at the genetic and phenotypic levels. The negative correlation observed between $LFEC_1$ and *Peps* ($r_p = -10$; $r_g = -0.51$) supports the hypothesis that at D_{+60}, most of the infestations were due to pre-adult, non-egg laying parasites. In a previous study carried out in adult Churra sheep, Martínez-Valladares *et al*. (2005a) observed a negative phenotypic correlation between the number of *T. circumcincta* eggs in the faeces and both the number of LIV and the concentration of plasma *Peps*, whereas a positive correlation was found between the number of LIV and the plasma *Peps* level. It seems, therefore, that in adult Churra sheep, the principal damage in GIN infections is due to the LIV stage. This is supported by the kinetics of the plasma *Peps* level observed in infections of Churra sheep, which reached their maximum around day 90 post-infection. In contrast, the maximum faecal egg excretion was observed approximately 150 to 160 days post-infection (J. Pérez, unpublished observations).

In lambs, several authors have reported negative genetic and environmental correlations between the FECs and the *IgA* level (Stear *et al*., 1997; Stear *et al*., 1999a and 1999b), which is in agreement with the role of IgA as a factor regulating the female parasite fecundity and inhibiting the adult parasite development. In our study, the plasma IgA level was not significantly correlated with the FEC measured on the same day (FEC_1), which supports the hypothesis proposed by Stear *et al*. (1999c).

It is also mentioning that Churra sheep is an autochthonous breed, well-adapted to the climatic and geographical conditions of the Castilla y León region. This breed is highly resistant to the effects of clinical infection and is able to maintain performance during GIN infection, which could be considered as resilience. In this regard, we calculated the genetic correlation between the parasite resistance traits studied here and milk yield (data not shown). The genetic correlations with milk traits were all negative and low (range: −0.08 to −0.18), but none were significant because of the large standard errors observed. T he phenotypic correlations were even weaker, and only one, between *Peps* and milk yield, was negative. Therefore, our data suggest that adult Churra sheep show resilience against GIN parasite infection. If this is confirmed, further studies should be carried out in Churra sheep to better understand the immune and genetic mechanisms underlying this interesting feature. However, these results should be taken with caution, as this experiment was not specifically designed to evaluate the effect of parasite infection on milk production.

In summary, this study demonstrated the presence of heritable variation in parasite resistance indicator traits in the Churra population studied. This suggests that genetic improvement could be possible for this complex feature in this population. However, further studies in which the experimental variables are controlled as much as possible are needed to identify the best traits for routine measurement of adult sheep to use as an indicator of parasite resistance.

References

Almería S, Llorente MM and Uriarte J 1996. Monthly fluctuations of worm burdens and hypobiosis of gastrointestinal nematodes of calves in extensive management systems in the Pyrenees (Spain). Veterinary Parasitology 67, 225–236.

Beraldi D, McRae AF, Gratten J, Pilkington JG, Slate J, Visscher PM and Pemberton JM 2007. Quantitative trait loci (QTL) mapping of resistance to strongyles and coccidia in the free-living Soay sheep (Ovis aries). International Journal for Parasitology 37, 121–129.

Bishop SC, Jackson F, Coop RL and Stear MJ 2004. Genetic parameters for resistance to nematode infections in Texel lambs and their utility in breeding programmes. Animal Science 78, 185–194.

Coop RL, Graham RB, Jackson F, Wright SE and Angus KW 1985. Effect of experimental Ostertagia circumcincta infection on the performance of grazing lambs. Research in Veterinary Science 38, 282–287.

Davies G, Stear MJ, Benothman M, Abuagob O, Kerr A, Mitchell S and Bishop SC 2006. Quantitative trait loci associated with parasitic infection in Scottish blackface sheep. Heredity 96, 252–258.

Davies G 2004. Investigating genetic aspects of the variation in the host response to gastrointestinal parasites in sheep. PhD, Glasgow University.

de la Fuente LF, Baro JA and San Primitivo F 1995. Breeding programme for the Spanish Churra sheep breed. Cahiers Options méditerranéennes 11, 165–172.

Donald AD 1979. Effects of parasites and disease on wool growth. In Physiological and environmental limitations to wool growth (ed. JL Black and PJ Reis), pp. 99–114. University of New England Publication, Armidale, NSW, Australia.

Edwards K, Jepson RP and Wood KF 1960. Value of plasma pepsinogen estimation. British Medical Journal 2, 30–31.

Gibbs HC and Barger IA 1986. Haemonchus contortus and other trichostrongylid infections in parturient, lactating and dry ewes. Veterinary Parasitology 22, 57–66.

Gray GD 1997. The use of genetically resistant sheep to control nematode parasitism. Veterinary Parasitology 72, 345–357.

Groeneveld E 1998. VCE user's guide, version 4.2. Institute of Animal Husbandry and Animal Sciences, Mariensee, Germany.

Houdijk JGM, Kyriazakis I, Jackson F, Huntley JF and Coop RL 2003. Is the allocation of metabolisable protein prioritised to milk production rather than to immune functions in Teladorsagia circumcincta infected lactating ewes? International Journal for Parasitology 33, 329–338.

Jeffcoate IA, Fishwick G, Bairden K, Armour J and Holmes PH 1990. Pathophysiology of the peri-parturient egg rise in sheep: the role of prolactin. Research in Veterinary Science 48, 295–300.

Kates KC 1947. Diagnosis of gastrointestinal nematode parasitism of sheep of some nematodes by differential egg counts. Proceedings of the Helminthological Society of Washington 14, 44–53.

Leyva V, Henderson AE and Sykes AR 1982. Effect of daily infection with Ostertagia circumcincta larvae on food intake, milk production and wool growth in sheep. Journal of Agricultural Science 99, 249–259.

Mackay AD, Betteridge K, Devantier BP, Budding PJ and Niezen J 1998. Chemical-free hill country sheep and beef livestock production systems. Proceedings of the New Zealand Grassland Association 60, 15–18.

Martínez-Valladares M, Vara-Del Río MP, Cruz-Rojo MA and Rojo-Vázquez FA 2005a. Genetic resistance to the infection by Teladorsagia circumcincta: IgA and parameters measured at slaughter in Churra sheep. Parasite Immunology 27, 213–218.

Martínez-Valladares M, Vara-del Río MP, Cruz-Rojo MA and Rojo-Vázquez FA 2005b. Effect of a low protein diet on the resistance of Churra sheep to Teladorsagia circumcincta. Parasite Immunology 27, 219–225(7).

Ministry of Agriculture Food and Fisheries 1986. Manual of Veterinary Parasitological Laboratory Techniques, 3rd edition. HMSO, London, UK.

Morris CA, Bisset SA, Vlassoff A, West CJ and Wheeler M 2004. Genetic parameters for *Nematodirus* spp. egg counts in Romney lambs in New Zealand. Animal Science 79, 33–39.

Murray M, Jennings FW and Armour J 1970. Bovine ostertagiasis: structure, function and mode of differentiation of the bovine gastric mucosa and kinetics of the worm loss. Research in Veterinary Science 11, 417–427.

Neumaier A and Groeneveld E 1998. Restricted maximum likelihood estimation of covariances in sparse linear models. Genetics Selection Evolution 30, 3–26.

Nieuwhof GJ and Bishop SC 2005. Costs of the major endemic diseases of sheep in Great Britain and the potential benefits of reduction in disease impact. Animal Science 81, 23–29.

Perry BD, Randolph RF, McDermott JJ, Sones KR and Thornton PK 2002. Investing in animal health research to alleviate poverty. International Livestock Research Institute (ILRI), Nairobi, Kenya, 148 pp.

Raadsma HW, Gray GD and Woolaston RR 1997. Genetics of disease resistance and vaccine response. In The genetics of sheep (ed. L Piper and A Ruvinsky), pp. 199–224. CABI Publishing, Oxon, UK.

Rojo-Vázquez FA 2004. The present status of helminth infections in small ruminants in Spain: prevalence, anthelmintic resistance an some considerations on their control. IX European multicolloquium of parasitology, Valencia, Spain, pp. 227–231.

SAS 1998. SAS user's guide: statistics, release 6.12. 1998. SAS Institute, Inc., Cary, NC.

Sayers G and Sweeney T 2005. Gastrointestinal nematode infection in sheep – a review of the alternatives to anthelmintics in parasite control. Animal Health Research Reviews 6, 159–171.

Stear MJ and Bishop SC 1999. The curvilinear relationship between worm length and fecundity of Teladorsagia circumcincta. International Journal for Parasitology 29, 777–780.

Stear MJ, Bairden K, Duncan JL, Gettinby G, McKellar QA, Murray M and Wallace DS 1995. The distribution of faecal nematode egg counts in Scottish Blackface lambs following natural, predominantly Ostertagia circumcincta infection. Parasitology 110, 573–581.

Stear MJ, Bairden K, Bishop SC, Buitkamp J, Duncan JL, Gettinby G, McKellar QA, Park M, Parkins JJ, Reid SW, Strain S and Murray M 1997. The genetic basis of resistance to Ostertagia circumcincta in lambs. Veterinary Journal 154, 111–119.

Stear MJ, Bairden K, McKeller QA, Scott I, Strain S and Bishop SC 1999a. The relationship between the number and size of nematodes in the abomasum and the concentration of pepsinogen in ovine plasma. Research in Veterinary Science 67, 89–92.

Stear MJ, Strain S and Bishop SC 1999b. How lambs control infection with Ostertagia circumcincta. Veterinary Immunology and Immunopathology 72, 213–218.

Stear MJ, Strain S and Bishop SC 1999c. Mechanisms underlying resistance to nematode infection. International Journal for Parasitology 29, 51–56.

Thomas RJ and Ali DA 1983. The effect of Haemonchus contortus infection on the pregnant and lactating ewe. International Journal for Parasitology 13, 393–398.

Waller PJ 1997. Anthelmintic resistance. Veterinary Parasitology 72, 391–412.

Windon RG 1996. Genetic control of resistance to helminths in sheep. Veterinary Journal for Immunology and Immunopathology 54, 245–254.

Woolaston RR and Baker RL 1996. Prospects of breeding small ruminants for resistance to internal parasites. International Journal for Parasitology 26, 845–855.