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Genetic analysis of the potential role of IgA and IgE responses against *Haemonchus contortus* in parasite resistance of Creole goats

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ABSTRACT

Small ruminants are affected by gastrointestinal nematode (GIN) infection. A promising alternative strategy for control of GIN infection is to increase the level of resistance in the population by taking advantage of the host's immune response. Immunoglobulin A (IgA) and E (IgE) are known to be involved in immune response to GIN. The aim of this study was thus to investigate genetic parameters of IgA and IgE responses against *Haemonchus contortus* in Creole kids naturally challenged at pasture and to determine the relationship with other resistance criteria such as faecal egg counts, packed-cell volume, eosinophil counts and bodyweight. Variance and covariance components for genetic and residuals effects for each trait were estimated on 3862 males at 11 months of age. Heritability estimates for IgA and IgE ranged between 0.15 and 0.57. Strong positive genetic correlations were observed between either IgE or IgA responses against L3 and adult excretory/secretory products (ESP) antigens of *H. contortus*, suggesting that the humoral immune response is not specific to the life cycle stage of the parasite suggesting that there is substantial cross recognition between the different parasite antigens. Heritability estimates for faecal egg count (FEC), packed-cell volume (PCV) and bodyweight (BW) were in accordance with previous results in Creole kids. Blood eosinophil counts were found moderately heritable and negatively correlated with FEC, suggesting that this cell population plays a role in resistance to nematode parasite infection in Creole goats. IgA response was positively correlated to FEC, in contrast with the negative correlation between IgE against L3 of *H. contortus* and FEC. In Creole goats, IgA response against L3 or ESP of *H. contortus* would rather be associated with the worm burden than an immune protective response. The immune response involving activity of IgE against L3 of *H. contortus* may be one important pathway for development of resistance to gastrointestinal nematode infections in Creole goats.

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1. Introduction

Gastrointestinal nematode (GIN) infections, particularly with *Haemonchus contortus*, cause major problems

for small ruminant production throughout the temperate and tropical regions of the world, with increasing cases of anthelmintic resistance (Sangster, 1999; Waller, 2003). One potential alternative strategy for control of GIN infection is to take advantage of the host's immune response in selection programs to increase the level of resistance in the population. Resistance is defined as the ability of an animal to limit the size of its worm population, that is to say, to prevent the installation of larvae and/or eliminate

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rapidly larvae and adults. Phenotypic criteria can be used to indirectly estimate resistance to parasites such as faecal egg counts, eosinophil counts, packed-cell volume or immunological traits (Dominik, 2005).

The immune response against GIN is mediated by recruitment of mucosal mast cells, globule leukocytes and eosinophils in digestive mucosa (Meeusen et al., 2005). The response against gastrointestinal nematodes also involves production of parasite-specific immunoglobulin A (IgA), IgG1 and IgE (Pfeffer et al., 1996; Kooyman et al., 1997; Shaw et al., 1998). Furthermore, increased expressions of immunoglobulin A and E are associated with resistance against GIN infection in sheep (Pernthaner et al., 2005, 2006). Data on the involvement of immunoglobulins in resistance to strongyles are scarce in goat. Moreover, little is known on the genetic control of IgA and IgE response against GIN in small ruminant.

Naturally contaminated tropical pastures have already been shown to allow the genetic evaluation of Creole goat resistance to gastrointestinal strongyles (Mandonnet et al., 2001, 2006). Creole goats come from the natural mixing of different breed imported from Africa, Europe and India during colonisation (Naves et al., 2001). This population has been naturally selected for its ability to resist harsh environment for about three centuries. Creole goats of the INRA-Gardel flock are indexed on their resistance under mixed natural infection conditions. The aims of this study were first to estimate the genetic parameters of immunoglobulin A and E responses against *H. contortus* and secondly their relationship with faecal egg counts, eosinophil counts, packed-cell volume and bodyweight.

2. Materials and methods

2.1. Experimental design

Animals used in this study came from the experimental flock of Creole goats reared at INRA-Gardel from 1995 to 2007 to study gastrointestinal nematode resistance. Animals are raised on pasture (*Digitaria decumbens*) all year long and naturally exposed to a mixed nematode infection. Main species infecting Creole goat are *H. contortus*, *Trichostrongylus colubriformis* and *Oesophagostomum columbianum*, in order of the importance of their prevalence (Aumont et al., 1997a). A high reproduction rhythm of three kiddings in two years was applied in the flock. Male kids from the same cohort were raised together and weaned at 82 days of age on average. Three different cohorts are raised each year. Anthelmintic treatment was administered every 8 weeks after weaning with levamisole (12 mg/kg).

2.2. Animal sampling

All traits were collected at 11 months of age. Faecal samples were collected two times (weeks 6 and 7 after drenching) to determine faecal egg counts (FEC) using a modified McMaster method for rapid determination (Aumont et al., 1997b). The FEC variable was the average of these two measures. Blood samples were also collected on week 6 after drenching for all animals. A sample was

centrifuged for 5 min at 5000 rpm and serum was then frozen at -20°C until analysis by indirect ELISA. Blood eosinophils (EOS) were counted according to the method of Dawkins et al. (1989), on Malassez cell counter. Packed cell volume (PCV) was also measured using the capillary micro-haematocrit method. Finally, bodyweight (BW) was measured at 325 days of age on average, corresponding approximately to 11 months of age.

2.3. Antibody responses by indirect ELISA

The activity of IgA and IgE against a crude extract of *H. contortus* L3 and excretory/secretory products (ESP) was measured by indirect ELISA according to Bambou et al. (2008). ESP were prepared using adult *H. contortus* collected from the abomasums of a sacrificed goat. Worms were washed in PBS (pH 7.4) with penicillin (100 IU/ml) and streptomycin (1 mg/ml) and maintained in this buffer by group of fifty adults in a 5% CO_2 atmosphere at 37°C overnight. The supernatant containing ESP were collected, filtered ($0.2\ \mu\text{m}$) and store at -80°C until further use. A crude extract of *H. contortus* L3 was prepared after three cycles of freezing and thawing (-80°C and $+25^{\circ}\text{C}$), homogenisation at 4°C and centrifugation at $30,000 \times g$ for 30 min at 4°C . The supernatant was used as the crude extract of the L3 antigen. The protein concentration of both antigenic products was determined with the method of Bradford (1976).

Antibodies against these two products were detected in serum using indirect ELISA. Antigens were diluted at $2\ \mu\text{g/ml}$ in carbonate buffer (pH 9.6) in 96-well plates (Nunc surface, Nunc, Denmark) and incubated overnight at 4°C . Wells were then washed three times with PBS with 0.1% Tween 20 (PBST). Non-specific binding sites were blocked by 3 h incubation with PBS-1% bovine serum albumin, 5% sucrose. Duplicate serum samples diluted 1:10 in PBST were incubated for 2 h at room temperature. Plates were washed three times with PBST before addition of horseradish peroxidase-conjugated rabbit anti-goat IgA (Rockland, PA, USA) diluted 1:5000 (IgA) in carbonate buffer. For IgE ELISA, plates were sequentially incubated with mouse anti-ovine IgE monoclonal IE7 (Kooyman et al., 1997) diluted 1:100,000 (provided by Dr. F.N.J. Kooyman, Utrecht University, The Netherlands) and rabbit anti-mouse horseradish peroxidase conjugate diluted 1:750 (Serotec, Oxford, England). After an incubation of 1 h at room temperature, plates were washed three times with PBST and $100\ \mu\text{l}$ of chromogen TMB (3,3', 5,5'-tetramethylbenzidine) was added in each well. The colour reaction was stopped by the addition of $50\ \mu\text{l}$ per well of $2\text{N H}_2\text{SO}_4$ and the optical density was measured at 450 nm (OD450). In order to compare results between assays, a positive control consisting of a pool of sera containing IgA and IgE antibodies was included on each plate. OD450 of tested serum were altered in proportion with changes of this standard. A negative control consisting of serum from a naive kid goat was also added on each plate.

2.4. Statistical analysis

Statistical analysis was made on 2288 male kids of 11 month of age for FEC, PCV, EOS and BW. IgA and IgE

response against L3 and ESP of *H. contortus* were analysed for 315 animals. Animals were born of 140 sires and 860 dams. Total pedigree of animals used in this study is composed of 249 sires and 1131 dams.

Distributions of traits were examined using a Shapiro–Wilk test and transformations were chosen to normalize the variance. FEC and EOS were log transformed using $\ln(\text{trait} + 15)$. PCV, BW, IgA and IgE response against L3 and ESP of *H. contortus* did not require any transformation. Fixed effects were fitted using the general linear model procedure of SAS software (SAS Institute Inc., 1999). Fixed effects tested for all traits were the cohort (39 levels), parity of dam (16 levels) and the type of nursing (natural or artificial), along with significant first order interactions. Phenotypic correlations r_p were calculated using the following formula (Minvielle, 1990):

$$r_p = r_a \sqrt{h_1^2 h_2^2} + r_e \sqrt{(1 - h_1^2)(1 - h_2^2)},$$

where r_a and r_e are genetic and residual correlations between trait 1 and trait 2 and h_1^2 and h_2^2 are heritability estimates for both traits. No standard errors are available for these correlations. The phenotypic correlation is the statistical relationship between two traits under the influence of genetic and environment factors. The genetic correlation is the statistical relationship between two traits under the influence of common genes. The relationship between these two correlations is not linear and may take opposite signs. Moreover, the genetic correlation is the most informative about the relationship between genes that control the mechanisms of the two traits.

Variance and covariance components for genetic and residuals effects were estimated using REML VCE package (Groeneveld, 1993), according to the following model:

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Za} + \mathbf{e}$$

where

$$\mathbf{E} \begin{bmatrix} \mathbf{a} \\ \mathbf{e} \end{bmatrix} = \begin{bmatrix} 0 \\ 0 \end{bmatrix} \text{ and } \text{Var} \begin{bmatrix} \mathbf{a} \\ \mathbf{e} \end{bmatrix} = \begin{bmatrix} \mathbf{G}_0 \otimes \mathbf{A} & 0 \\ 0 & \mathbf{R}_0 \otimes \mathbf{I} \end{bmatrix}$$

where $\mathbf{y}$ is the vector of observations, $\mathbf{b}$ is the vector of significant fixed effects, $\mathbf{a}$ is the vector of random direct additive genetic effects, $\mathbf{e}$ is the vector of random residual effects, $\mathbf{X}$ and $\mathbf{Z}$ are the incidence matrices connecting the $\mathbf{y}$ to the effects in the model, $\mathbf{G}_0$ is the additive genetic variance-covariance matrix between traits, $\mathbf{R}_0$ is the residual variance-covariance matrix between traits, $\mathbf{A}$ is the relationship matrix amongst animals and $\mathbf{I}$ is the identity matrix. The variances of the random effects $\mathbf{a}$ and $\mathbf{e}$ are σ_a^2 , σ_e^2 . Maternal effects were not significant for BW, FEC, PCV and EOS. We failed to include them for analyses of IgA and IgE responses against L3 and ESP of *H. contortus* due to the size of the data set.

A first multivariate model was used to estimate genetic parameters of IgA and IgE responses against L3 and ESP of *H. contortus*. Those traits were then fitted separately on two other multivariate models to obtain the genetic correlations with other traits (FEC, EOS, PCV and BW).

3. Results

3.1. Descriptive statistics

The least-square means, range and standard deviation for all traits are presented in Table 1. In average the infection level of kids was about 1300 epg. Ranges observed for all traits analysed showed a wide variation amongst the population. Levels of IgE and IgA responses were higher against L3 than ESP antigens with as shown by higher means.

3.2. Genetic parameters

Significant fixed effects for FEC, EOS and BW were the cohort, rank of litter and the type of nursing. Significant fixed effects for PCV were the cohort and the type of nursing. The cohort was the only significant fixed effect for all IgE and IgA traits.

A positive phenotypic correlation was found between IgE anti-ESP and IgE anti-L3 and between IgA anti-ESP and IgA anti-L3 (Table 2). Negative phenotypic correlations were found between FEC and EOS, PCV and BW (Table 3). IgE anti-ESP was negatively correlated with FEC and positively correlated with PCV. EOS was positively correlated with IgA anti-ESP and IgA anti-L3 (Table 3a and b).

Heritability estimates for IgA and IgE responses against L3 and ESP of *H. contortus* are presented in Table 2. Heritability estimates for other traits are presented in Table 3a and b. Heritability and correlation estimates were consistent between the three models. Non significant differences were due to sampling effects. Parameters of FEC, PCV and BW are an update of those previously estimated (Mandonnet et al., 2001). Their heritability estimates ranged between 0.21 and 0.31. Heritability estimated in this present study for EOS on 11 month-old kids was higher (0.20 ± 0.03). The IgE anti-ESP, IgA anti-ESP and IgA anti-L3 responses were moderately heritable whereas the IgE anti-L3 response appears to be highly heritable (0.57 ± 0.16). However, the standard error for this estimate was higher compared to the other traits (0.10 in average).

Genetic correlations between IgA and IgE responses against L3 and ESP of *H. contortus* are shown above the diagonal in Table 2. Genetic correlations within FEC, PCV, EOS and BW and between these traits and IgE and IgA responses against L3 and ESP of *H. contortus* are presented above the diagonal in Table 3a and b. FEC was negatively correlated with PCV. FEC was also negatively correlated with EOS. PCV and BW were positively correlated as previously estimated (Mandonnet et al., 2001). The IgE anti-ESP and IgE anti-L3 were strongly and positively correlated as shown by a genetic correlation of almost 1 (0.99 ± 0.01). A strong relationship was also found between IgA anti-L3 and IgA anti-PES showing a genetic correlation of 0.89 ± 0.10 .

Genetic correlations between IgE anti-ESP, IgE anti-L3 and FEC, PCV, EOS and BW (Table 3a) showed quite high standard errors (0.10 on average (0.06 – 0.21)). Nevertheless, IgE anti-L3 was significantly negatively correlated to FEC and positively correlated to BW (-0.32 ± 0.08 and 0.35 ± 0.16 respectively). No significant correlation between IgE anti-ESP response and classical traits were

Table 1

Descriptive statistics of traits related to parasite resistance.

Trait	n	Mean	Range	Standard deviation
FEC (ln eggs/g + 15)	1835	6.28 (1291)	2.71–9.99 (0–21,690)	1.49 (1988)
EOS (ln cells/ml + 15)	1596	6.84 (1427)	2.71–9.37 (0–11,693)	1.05 (1347)
PCV (%)	1730	25.87	10–40	4.65
BW (kg)	1993	17.91	6.67–32.85	3.75
IgE anti-ESP (OD ratio)	268	0.14	0.03–0.33	0.05
IgE anti-L3 (OD ratio)	271	0.28	0.04–0.60	0.12
IgA anti-ESP (OD ratio)	261	0.22	0.03–0.65	0.14
IgA anti-L3 (OD ratio)	260	0.32	0.03–0.74	0.19

Means, range and standard deviation of non transformed data are showed in brackets. Different subsets of animal were involved for different traits.

Table 2Phenotypic and genetic parameters of IgA and IgE responses against L3 and ESP of *H. contortus*.

	IgE anti-ESP	IgE anti-L3	IgA anti-ESP	IgA anti-L3
IgE anti-ESP	0.23 (0.12)	0.99 (0.01)	−0.43 (0.28)	0.02 (0.41)
IgE anti-L3	0.61	0.57 (0.16)	−0.51 (0.26)	−0.07 (0.39)
IgA anti-ESP	0.06	−0.005	0.27 (0.11)	0.89 (0.10)
IgA anti-L3	0.09	0.09	0.82	0.15 (0.11)

Phenotypic correlations are presented below the diagonal, heritability estimates on diagonal and genetic correlations above the diagonal. Standard deviations are shown between brackets. Parameters were estimated on a subset of 315 kids.

found. In contrast, IgA anti-ESP and IgA anti-L3 were strongly positively correlated with FEC despite high standard errors (Table 3b). IgA anti-ESP response was negatively correlated with PCV and BW (-0.36 ± 0.18 and -0.50 ± 0.15 , respectively) whereas IgA anti-L3 response was negatively correlated with EOS and BW (-0.44 ± 0.25 and -0.38 ± 0.16 , respectively).

4. Discussion

In this study, we quantified the genetic parameters of parasite resistance traits of Creole kids at 11 months of age, under natural mixed infection with *H. contortus* and *T. colubriformis* predominantly. We measured FEC, PCV, EOS, BW and serum parasite-specific IgA and IgE responses. These traits are considered as different criteria related to the pathophysiology of GIN infection (Dominik, 2005).

Significant heritability estimates for FEC, PCV, EOS and BW ranged from 0.20 to 0.31, and are in the range of heritability estimates reported in sheep (Bishop et al., 2004;

Vanimisetti et al., 2004; Morris et al., 2005; McManus et al., 2009; Prince et al., 2010) and in goats (Baker et al., 2001; Vagenas et al., 2002). Heritability estimates for FEC and PCV were in accord with our previous data in Creole goats with a range of 0.14–0.33 and 0.10–0.33 respectively (Mandonnet et al., 2001). However, lower estimates were also observed in other goat breeds (Morris et al., 1997; Mandal et Sharma, 2008; Bolormaa et al., 2010) as well as in sheep breeds (Rege et al., 2002; Wolf et al., 2008). Our greater heritability estimates in Creole goats, compared to other goat breeds in tropics, may be due to the relatively standardized experimental conditions and flock management in Guadeloupe (Mandonnet et al., 2001).

The heritability estimates for EOS and IgA responses were in the same range than other published estimates in goat (Bolormaa et al., 2010; Gutiérrez-Gil et al., 2010). Heritability estimates for EOS were in accordance with our previous data (Mandonnet et al., 2006) under natural mixed infection condition of Creole does (ranged 0.12–0.24, recorded at week 4 and week 6 after kidding respectively).

Table 3Phenotypic and genetic correlations between resistance, production traits and IgE response (a) and IgA response (b) against L3 and ESP of *H. contortus* (estimations may slightly differ between (a) and (b) due to the different sets of variables included in the multivariate model).

	FEC	EOS	PCV	BW	IgE anti-ESP	IgE anti-L3
(a)						
FEC	0.21 (0.03)	−0.23 (0.06)	−0.35 (0.08)	−0.007 (0.06)	−0.08 (0.12)	−0.32 (0.08)
EOS	−0.11	0.20 (0.03)	−0.05 (0.07)	−0.09 (0.07)	−0.18 (0.14)	0.03 (0.09)
PCV	−0.24	0.03	0.22 (0.03)	0.40 (0.07)	0.18 (0.12)	−0.09 (0.06)
BW	−0.10	0.04	0.49	0.31 (0.03)	0.13 (0.21)	0.35 (0.16)
IgE-ESP	−0.14	−0.06	0.13	0.08	0.18 (0.10)	0.79 (0.11)
IgE-L3	−0.08	−0.03	−0.01	0.07	0.60	0.44 (0.13)
(b)						
FEC	0.20 (0.03)	−0.22 (0.08)	−0.35 (0.08)	0.0005 (0.08)	0.84 (0.13)	0.72 (0.18)
EOS	−0.11	0.20 (0.04)	−0.04 (0.10)	−0.07 (0.12)	−0.005 (0.31)	−0.44 (0.25)
PCV	−0.24	0.03	0.22 (0.03)	0.39 (0.08)	−0.36 (0.18)	0.04 (0.17)
BW	−0.10	0.04	0.49	0.32 (0.04)	−0.50 (0.15)	−0.38 (0.16)
IgA-ESP	−0.06	0.10	0.03	−0.02	0.19 (0.09)	0.83 (0.11)
IgA-L3	−0.04	0.10	0.004	−0.02	0.82	0.23 (0.11)

Phenotypic correlations are presented below the diagonal, heritability estimates on diagonal and genetic correlations above the diagonal. Standard deviations are shown between brackets. Parameters were estimated on the whole dataset.

In Australian Angora goat kids at 3 and 5 months of age and 35 days following an experimental infection with *T. colubriformis*, heritability estimates for EOS ranged from 0.06 to 0.28 (Bolormaa et al., 2010). However, higher heritability for EOS was found by Davies et al. (2005) in 6 month-old lambs naturally exposed to a mixed nematode infection and in 4–5 month old Scottish blackface lambs (Stear et al., 2002). To the best of our knowledge, no other study was published in goat on genetic parameters of IgA and IgE responses against *H. contortus*. Recently, Bolormaa et al. (2010) investigated genetic parameters of another immunoglobulin, IgG, against *T. Colubriformis* and found heritability estimates ranging from 0.14 to 0.42. In Creole kids, heritability estimates for IgA against *H. contortus* are lower than those reported in Scottish blackface lambs, Romney lambs and Spanish Churra ewes (Davies et al., 2005; Gutiérrez-Gil et al., 2010) but in the same range than those published by Davies et al. (2006). However, in Creole kids, the highest heritability estimate was for IgE anti-L3 (0.57 ± 0.16), a value in the range of those estimated in sheep against *T. colubriformis* and *Teladorsagia circumcincta* (Shaw et al., 1999; Murphy et al., 2010). Thus, significant genetic variability was observed in Creole goats for traits associated with the host immune response: low to high heritability estimates for IgA and IgE responses against L3 and ESP of *H. contortus* and EOS. Therefore, these traits could be proposed as criteria of resistance to GIN in a breeding scheme. However, before selecting animals on these traits to improve resistance to GIN, they should not be in an antagonistic relation with production traits.

In Creole goats, the genetic correlation between EOS and BW was not significant. Furthermore, the negative genetic correlation found between EOS and FEC suggests that this cell population plays a role in resistance to helminth infection. A similar but stronger correlation (-0.62 ± 0.20) between resistance to *T. colubriformis* infection and the magnitude of the peripheral eosinophil response has been shown in resistant line of Merino sheep (Woolaston et al., 1996). As EOS is easier and cheaper to measure than FEC, one could imagine replacing FEC by EOS as selection criteria. However, Woolaston et al. (1996) reported that the efficiency of an indirect selection based on EOS is only 43% relative to direct selection on FEC. In our case the genetic progress with indirect selection on EOS would be 48% of the one of direct selection on eggs output (Mandonnet personal communication). Moreover, we realized simulations on the expected genetic progress of FEC (SelAction software, Rutten et al., 2002) by combining EOS and FEC. The expected genetic progress of FEC in this case was not significantly improved by adding a criterion reflecting the host immune mechanisms (unpublished data). Therefore, selection for resistance would be optimal by measuring FEC only.

The strong positive genetic correlations between either IgE or IgA responses against L3 and adult ESP antigens of *H. contortus* suggest that the humoral immune response is not specific to the life cycle stage of the parasite probably due to substantial cross recognition between the different parasite antigens. A high positive genetic correlation was observed between the IgA response and FEC. Our data suggest that in Creole goats, the IgA response reflects the size of the nematode burden (assessed by FEC) rather than the

efficiency of the protective immune response. In contrast, previous studies in sheep reported negative genetic and phenotypic correlations between FEC and the IgA response (Amarante et al., 2005; Davies et al., 2005) in agreement with the role of IgA as the major mechanism controlling GIN fecundity and adult development (Stear et al., 1995; Strain et al., 2002). Furthermore, the cost of producing these immune responses against gastrointestinal parasite in Creole goats surpasses the benefits of the protection provided by these responses as a negative genetic correlation was observed between the IgA response and BW. Resources allocation is thus in competition between growth and immune response. In contrast, activity of IgA is not only ineffective in controlling nematode infection but also is in direct competition with growth as shown by the negative genetic correlation with BW.

The IgE anti-ESP response showed no genetic correlation with the different traits analysed in this study. Moreover, a weak and negative phenotypic correlation was observed between IgE anti-ESP and FEC, in contrast with previous results in Creole kids showing a positive phenotypic correlation between these two traits (Bambou et al., 2008). The environments (on pasture in our study and indoors preceding) and the two infection modes (natural vs. experimental) may explain this difference. Inversely, the IgE anti-L3 responses showed a negative genetic correlation with FEC and a positive genetic correlation with BW. These findings are consistent with previous results suggesting involvement of this antibody isotype in protection against GIN infection in sheep (Shaw et al., 1998; Pettit et al., 2005; Murphy et al., 2010). To the best of our knowledge, parasite-specific IgE play a central role in immediate hypersensitivity reactions, by cross-linking of IgE on the mast cell surface. Based on heritability estimates and genetic correlations found in this study, the immediate hypersensitivity reaction mediated by IgE against L3 of *H. contortus* would be one key point of the immune response in Creole goats. These protective mechanisms are not pathogenic, in contrast to Murphy et al. (2010), as shown by the positive genetic correlation between IgE anti-L3 and BW.

In summary, even if IgE and IgA effectively recognize both L3 and adults ESP antigens, all these responses would not result in a protective reaction for the host. Immunoglobulin A recognizes L3 and adults as suggested by the positive genetic correlations with FEC but this would not result in effective reduction of the parasite population in Creole goats. In other hand, the immune response involving IgE directed against L3 of *H. contortus* would be efficient to control the nematode population, as suggested by the negative genetic correlation with FEC. However, the protective response would not to be directed against adults of *H. contortus* as suggested by the weak genetic correlation with FEC. As mentioned above, the immune response involving activity of IgE against L3 of *H. contortus* may be one important pathway for development of resistance to gastrointestinal nematode infections in Creole goats (Schallig, 2000).

Eosinophil cells, another immunological trait studied (Robinson et al., 2010), may reduce efficiently the nematode population as this trait is negatively correlated with

FEC. Besides, EOS was also negatively correlated with IgA anti-L3. As the response of IgA anti-L3 may reflect directly the presence of L3 in the digestive system of the animal, this suggests that activity of eosinophil cells would be directed toward L3 rather than adults. Intimate contact between *H. contortus* L3 and eosinophils has been evidenced by Terefe et al. (2007) in blackbelly sheep. Moreover, presence of antibodies and complement seems to be essential for adherence and immobilisation of larvae by eosinophils (Balic et al., 2006; Terefe et al., 2007). In our study, IgA, correlated to the nematode burden, could act as a complement and may enhance the larval killing potential of blood eosinophils. However, the absence of genetic correlation with IgE response suggests that the immune response involving eosinophil cells may be independent of the immune response involving IgE. These two pathways may be different and may offer two effective and protective responses to nematode infection in Creole goats.

Similar immune pathways directed toward L3 only were not demonstrated in sheep. The immune response in sheep seems to be more complex as it is also directed against adults. This evidenced the general hypothesis that goats, as their contact with parasites are more recent due to their feeding behaviour, have developed a more simple protective response against nematode parasite by targeting the L3, first stage in contact with animals.

5. Conclusion

This study demonstrated that IgA and IgE against L3 and adults of *H. contortus* are under genetic control in Creole goats. Moreover, IgA responses were genetically positively correlated with FEC whereas IgE responses and EOS were negatively correlated with FEC. This study provides interesting results and formulates hypothesis on mechanisms of resistance involved in goats. Further research will attempt to explore local response of IgA and IgE in mucus.

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References

- Amarante, A.F.T., Bricarello, P.A., Huntley, U., Mazzolin, L.P., Gomes, J.C., 2005. Relationship of abomasal histology and parasite-specific immunoglobulin A with the resistance to *Haemonchus contortus* infection in three breeds of sheep. *Vet. Parasitol.* 128, 99–107.
- Aumont, G., Pouillot, R., Simon, R., Hostache, G., Varo, H., Barre, N., 1997a. Parasitisme digestif des petits ruminants dans les Antilles Françaises. *Inra Prod. Anim.* 10, 79–89.
- Aumont, G., Pouillot, R., Mandonnet, N., Nzobadila, G., 1997b. Le dénombrement des éléments parasitaires: Un outil pour l'étude de la résistance génétique aux endo-parasites chez les petits ruminants. Workshop final de l'ATP CIRAD-MIPA 72/94, Guadeloupe, France.
- Baker, R.L., Audho, J.O., Aduda, E.O., Thorpe, W., 2001. Genetic resistance to gastro-intestinal nematode parasites in Galla and Small East African goats in the sub-humid tropics. *Anim. Sci.* 73, 61–70.
- Balic, A., Cunningham, C.P., Meeusen, E.N.T., 2006. Eosinophil interactions with *Haemonchus contortus* larvae in the ovine gastrointestinal tract. *Parasite Immunol.* 28, 107–115.
- Bambou, J.-C., de la Chevrotière, C., Varo, H., Arquet, R., Kooyman, F.N.J., Mandonnet, N., 2008. Serum antibody responses in Creole kids experimentally infected with *Haemonchus contortus*. *Vet. Parasitol.* 158, 311–318.
- Bishop, S.C., Jackson, F., Coop, R.L., Stear, M.J., 2004. Genetic parameters for resistance to nematode infections in Texel lambs and their utility in breeding programmes. *Anim. Sci.* 78, 185–194.
- Bolormaa, S., Olayemi, M., van der Werf, J.H.J., Baillie, N., Le Jambre, F., Ruvinsky, A., Walkden-Brown, S.S., 2010. Estimates of genetic and phenotypic parameters for production, haematological and gastrointestinal nematode-associated traits in Australian Angora goats. *Anim. Prod. Sci.* 50, 25–36.
- Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein–dye binding. *Anal. Biochem.* 72, 248–254.
- Davies, G., Stear, M.J., Bishop, S.C., 2005. Genetic relationships between indicator traits and nematode parasite infection levels in 6-month-old lambs. *Anim. Sci.* 80, 143–150.
- Davies, G., Stear, M.J., Benothman, M., Abuagob, O., Kerr, A., Mitchell, S., Bishop, S.C., 2006. Quantitative trait loci associated with parasitic infection in Scottish blackface sheep. *Heredity* 96, 252–258.
- Dawkins, H.J.S., Windon, R.G., Eagleson, G.K., 1989. Eosinophil responses in sheep selected for high and low responsiveness to *Trichostrongylus colubriformis*. *Int. J. Parasitol.* 19, 199–205.
- Dominik, S., 2005. Quantitative trait loci for internal nematode resistance in sheep: a review. *Genet. Sel. Evol.* 37, 83–96.
- Groeneveld, E., 1993. REML-VCE a multivariate model restricted maximum likelihood (co)variance component estimation package. In: Groeneveld, E. (Ed.), *Proc. EC Symp. on Appl. of Mixed Linear Model in the Predictive of Genetic Merit in Pigs*. Mariensee, Germany, pp. 83–102.
- Gutiérrez-Gil, B., Pérez, J., de la Fuente, L.F., Meana, A., Martínez-Valladares, M., San Primitivo, F., Rojo-Vazquez, F.A., Arranz, J.J., 2010. Genetic parameters for resistance to trichostrongylid infection in dairy sheep. *Animal* 4, 505–512.
- Kooyman, F.N.J., VanKooten, P.J.S., Huntley, J.F., MacKellar, A., Cornelissen, A.W.C.A., Schallig, H.D.F.H., 1997. Production of a monoclonal antibody specific for ovine immunoglobulin E and its application to monitor serum IgE responses to *Haemonchus contortus* infection. *Parasitology* 114, 395–406.
- Mandal, A., Sharma, D.K., 2008. Inheritance of faecal nematode egg count in Barbari goats following natural *Haemonchus contortus* infection. *Vet. Parasitol.* 155, 89–94.
- Mandonnet, N., Aumont, G., Fleury, J., Arquet, R., Varo, H., Gruner, L., Bouix, J., Khang, J.V.T., 2001. Assessment of genetic variability of resistance to gastrointestinal nematode parasites in Creole goats in the humid tropics. *J. Anim. Sci.* 79, 1706–1712.
- Mandonnet, N., Menendez-Buxadera, A., Arquet, R., Mahieu, M., Bachand, M., Aumont, G., 2006. Genetic variability in resistance to gastrointestinal strongyles during early lactation in Creole goats. *Anim. Sci.* 82, 283–287.
- McManus, C., Louvandini, H., Paiva, S.R., de Oliveira, A.A., Azevedo, H.C., de Melo, C.B., 2009. Genetic factors of sheep affecting gastrointestinal parasite infections in the Distrito Federal, Brazil. *Vet. Parasitol.* 166, 308–313.
- Meeusen, E.N.T., Balic, A., Bowles, V., 2005. Cells, cytokines and other molecules associated with rejection of gastrointestinal nematode parasites. *Vet. Immunol. Immunopathol.* 108, 121–125.
- Minville, F. (Ed.), 1990. *Principes d'amélioration génétique des animaux domestiques*. Les presses de l'Université Laval, Laval, p. 211.
- Morris, C.A., Wheeler, M., Hosking, B.C., Watson, T.G., Hurford, A.P., Foote, B.J., Foote, J.F., 1997. Genetic parameters for milk yield and faecal nematode egg count in Saanen does. *N. Z. J. Agric. Res.* 40, 523–528.
- Morris, C.A., Wheeler, M., Watson, T.G., Hosking, B.C., Leathwick, D.M., 2005. Direct and correlated responses to selection for high or low faecal nematode egg count in Perendale sheep. *N. Z. J. Agric. Res.* 48, 1–10.
- Murphy, L., Echersall, P.D., Bishop, S.C., Pettit, J.J., Huntley, J.F., Burchmore, R., Stear, M.J., 2010. Genetic variation among lambda in peripheral IgE activity against the larval stages of *Teladorsagia circumcincta*. *Parasitology* 137, 1249–1260.

- Naves, M., Alexandre, G., Leimbacher, F., Mandonnet, N., Menendez-Buxadera, A., 2001. Les ruminants domestiques de la Caraïbe: le point sur les ressources génétiques et leur exploitation. *Inra Prod. Anim.* 14, 181–192.
- Pernthaner, A., Shaw, R.J., McNeill, M.M., Morrison, L., Hein, W.R., 2005. Total and nematode-specific IgE responses in intestinal lymph of genetically resistant and susceptible sheep during infection with *Trichostrongylus colubriformis*. *Vet. Immunol. Immunopathol.* 104, 69–80.
- Pernthaner, A., Cole, S.A., Morrison, L., Green, R., Shaw, R.J., Hein, W.R., 2006. Cytokine and antibody subclass responses in the intestinal lymph of sheep during repeated experimental infections with the nematode parasite *Trichostrongylus colubriformis*. *Vet. Immunol. Immunopathol.* 114, 135–148.
- Pettit, J.J., Jackson, F., Rocchi, M., Huntley, J.F., 2005. The relationship between responsiveness against gastrointestinal nematodes in lambs and the numbers of circulating IgE-bearing cells. *Vet. Parasitol.* 134, 131–139.
- Prince, L.L.L., Gowane, G.R., Swarnkar, C.P., Singh, D., Arora, A.L., 2010. Estimated of genetic parameters for faecal egg count of *Haemonchus contortus* infection and relationship with growth traits in Avikalin sheep. *Trop. Anim. Health Prod.* 42, 785–791.
- Pfeffer, A., Douch, P.G.C., Shaw, R.J., Gatehouse, T.K., Rabel, B., Green, R.S., Shirer, C.L., Jonas, W.E., Bisset, S., 1996. Sequential cellular and humoral responses in the abomasal mucosa and blood of Romney sheep dosed with *Trichostrongylus axei*. *Int. J. Parasitol.* 26, 765–773.
- Rege, J.E.O., Tembely, S., Mukasa-Mugerwa, E., Sovani, A., Anindo, D., Lahlou-Kassi, A., Nagda, S., Baker, R.L., 2002. Effect of breed and season on production and response to infection with gastro-intestinal nematode parasites in sheep in the highlands of Ethiopia. *Livest. Prod. Sci.* 78, 159–174.
- Robinson, N., Pieddrafit, D., Snibson, K., Harrison, P., Meeusen, E.N., 2010. Immune cell kinetics in the ovine abomasal mucosa following hyperimmunization and challenge with *Haemonchus contortus*. *Vet. Res.* 41, 37.
- Rutten, M.J.M., Bijma, P., Woolliams, J.A., van Arendonk, J.A.M., 2002. SelAction: software to predict selection response and rate of inbreeding in livestock breeding programs. *J. Hered.* 93, 456–458.
- Sangster, N.C., 1999. Anthelmintic resistance: past, present and future. *Int. J. Parasitol.* 29, 115–124.
- SAS, 1999. SAS Release 8.1. SAS Institute Inc., Cary, NC, USA.
- Schallig, H.D.F.H., 2000. Immunological responses of sheep to *Haemonchus contortus*. *Parasitology* 120, S63–S72.
- Shaw, R.J., Gatehouse, T.K., McNeill, M.M., 1998. Serum IgE responses during primary and challenge infections of sheep with *Trichostrongylus colubriformis*. *Int. J. Parasitol.* 28, 293–302.
- Shaw, R.J., Morris, C.A., Green, R.S., Wheeler, M., Bisset, S.A., Vlasoff, A., Douch, P.G.C., 1999. Genetic and phenotypic relationships among *Trichostrongylus colubriformis*-specific immunoglobulin E, anti-*Trichostrongylus colubriformis* antibody, immunoglobulin G(1), faecal egg count and body weight traits in grazing Romney lambs. *Livest. Prod. Sci.* 58, 25–32.
- Stear, M.J., Bishop, S.C., Doligalska, M., Duncan, J.L., Holmes, P.H., Irvine, J., McCririe, L., McKellar, Q.A., Sinski, E., Murray, M., 1995. Regulation of egg production, worm burden, worm length and worm fecundity by host responses in sheep infected with *Ostertagia circumcincta*. *Parasite Immunol.* 17, 643–652.
- Stear, M.J., Henderson, N.G., Kerr, A., McKellar, Q.A., Mitchell, S., Seeley, C., Bishop, S.C., 2002. Eosinophilia as a marker of resistance to *Teladorsagia circumcincta* in Scottish blackface lambs. *Parasitology* 124, 553–560.
- Strain, S.A.J., Bishop, S.C., Henderson, N.G., Kerr, A., McKellar, Q.A., Mitchell, S., Stear, M.J., 2002. The genetic control of IgA activity against *Teladorsagia circumcincta* and its association with parasite resistance in naturally infected sheep. *Parasitology* 124, 545–552.
- Terefe, G., Grisez, C., Prevot, F., Bergeaud, J.P., Dorchie, P., Brunel, J.C., François, D., Fourquaux, I., Jacquet, P., 2007. In vitro pre-exposure of *Haemonchus contortus* L3 to blood eosinophils reduces their establishment potential in sheep. *Vet. Res.* 38, 647–654.
- Vagenas, D., Jackson, F., Russel, A.J.F., Merchant, M., Wright, I.A., Bishop, S.C., 2002. Genetic control of resistance to gastro-intestinal parasites in crossbred cashmere-producing goats: responses to selection, genetic parameters and relationships with production traits. *Anim. Sci.* 74, 199–208.
- Vanimisetti, H.B., Andrew, S.L., Zajac, A.M., Notter, D.R., 2004. Inheritance of fecal egg count and packed cell volume and their relationship with production traits in sheep infected with *Haemonchus contortus*. *J. Anim. Sci.* 82, 1602–1611.
- Waller, P.J., 2003. Global perspectives on nematode parasite control in ruminant livestock: the need to adopt alternatives to chemotherapy, with emphasis on biological control. *Anim. Health Res. Rev.* 4, 35–43.
- Wolf, B.T., Howells, K., Nakielnny, C., Haresign, W., Lewis, R.M., Davies, O., Davies, M.H., 2008. Genetic parameters for strongyle and *Nematodirus* faecal egg counts in lambs and their relationships with performance traits. *Livest. Sci.* 113, 209–217.
- Woolaston, R.R., Manuelli, P., Eady, S.J., Barger, I.A., Le Jambre, L.F., Banks, D.J.D., Windon, R.G., 1996. The value of circulating eosinophil count as a selection criterion for resistance of sheep to trichostrongyle parasites. *Int. J. Parasitol.* 26, 123–126.