Veterinary Parasitology 264 (2018) 18–25

Contents lists available at ScienceDirect

Veterinary Parasitology

journal homepage: www.elsevier.com/locate/vetpar

Research paper

Boer goats appear to lack a functional IgA and eosinophil response against natural nematode infection

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ARTICLE INFO

Keywords:
Sheep
Nematode
Teladorsagia circumcincta
Immune response
IgA
Eosinophil

ABSTRACT

Gastrointestinal nematode infection is one of the major diseases affecting small ruminants. Although some breeds of goats are quite resistant, many breeds of goats are relatively susceptible. This study used a combined parasitological, immunological, bioinformatic and statistical approach to examine the role of goat IgA and eosinophils in protection against *Teladorsagia circumcincta*. Molecular modelling suggested that the transmembrane domain of the high affinity IgA receptor was dysfunctional in goats. Statistical analyses failed to find any association in naturally infected goats between high IgA or eosinophil responses and low faecal egg counts. Together these results indicate that IgA and eosinophil responses against *T. circumcincta* are less effective in goats than sheep.

1. Introduction

Gastrointestinal nematodes (GIN) pose an existential threat to small ruminants such as sheep and goats (Hoste et al., 2010). The two species have evolved different strategies to minimise the threat (Hoste et al., 2008). In sheep, the immune response is the major mechanism of defence (McRae et al., 2015). The nematode *Teladorsagia circumcincta* is the dominant nematode in cool temperate areas of the world and possibly there is more known about the mechanisms of protective immunity to this nematode than any other (Stear et al., 1995). There are three major signs of immunity (Smith et al., 1984). The first is reduced establishment and survival which is strongly associated with a local type 1 hypersensitivity response (Miller, 1984; Murphy et al., 2010; Stear et al., 1995). The second sign of resistance is an increased number of inhibited larvae which has been weakly associated with a local IgA response (Smith et al., 1985; Strain et al., 2002) although there is some doubt as to whether the IgA response is quick enough to influence larval inhibition (Halliday et al., 2010). Further, larval inhibition can also be influenced by density-dependent effects (Dunsmore, 1961) as well as season and larval strain (Bairden, personal communication). The third sign of immunity is reduced adult size and fecundity which is strongly associated with the local IgA and eosinophil response (Henderson and Stear, 2006; Stear et al., 1995). The ability to control worm growth develops before the ability to control worm number (Seaton et al., 1989; Stear et al., 1995).

In contrast, goats prefer to browse rather than graze and this limits nematode infection (Hoste et al., 2010). Goats that are kept with sheep and allowed to browse often have lower egg counts than sheep (Hoste et al., 2008) while goats that co-graze with sheep often have higher faecal nematode egg counts (FEC) (Le Jambre and Royal, 1976). The higher egg counts in co-grazed goats has led to the hypothesis that the immune response to GIN is less efficient in goats than sheep (Le Jambre and Royal, 1976). The ability to reduce larval establishment and survival is weaker in goats (Huntley et al., 1995). Relatively resistant goats have more inhibited larvae than relatively susceptible goats (Paterson et al., 1996a, b) and, possibly as a consequence of going into temporary inhibition, worms can develop more slowly (Huntley et al., 1995).

Strong parasite-specific IgA responses are associated with decreased FEC in Scottish Blackface (Strain et al., 2002) and in Santa Ines, Suffolk and Ile de France sheep (Amarante et al., 2005). In Scottish Cashmere goats that were selectively bred for improved nematode resistance, high parasite-specific IgA responses were associated with high FEC. In the same study, there was only a weak correlation between eosinophilia and FEC in the selected animals although their peripheral eosinophil

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https://doi.org/10.1016/j.vetpar.2018.10.014
Received 5 July 2018; Received in revised form 18 October 2018; Accepted 20 October 2018

counts were greater than the control animals (McBean et al., 2016). Similarly in Creole goats, faecal egg count was positively correlated with IgA but was negatively correlated with peripheral eosinophil count (de la Chevrotière et al., 2012) even though this breed can be bred for improved resistance to gastrointestinal nematodes (Mandonnet et al., 2001). The relationships of FEC with IgA and eosinophilia are unknown in Boer goats.

In humans, eosinophils express a single receptor for IgA, CD89 the high affinity IgA receptor. Mice lack the IgA receptor (van Egmond et al., 2001) and their eosinophils do not influence protection against some nematodes (Urban et al., 1991). The purpose of this paper was to examine the role of goat IgA and eosinophils in protection against *T. circumcincta* with a combined parasitological, immunological, bioinformatic and statistical approach.

2. Materials and methods

2.1. Bioinformatic analysis of the high affinity IgA receptor in goats, sheep and humans

The amino acid sequence for human CD89 was retrieved from the NCBI website and used as the query sequence for BLAST searches against *Ovis aries* and *Capra hircus* protein sets. Two sequences from *O. aries* and one sequence from *C. hircus* were retrieved. Both of the retrieved sequences for *O. aries* were identical apart from a deletion of 12 amino acids from position 12–23; this was presumed to be prediction error from the algorithm used to identify hypothetical proteins and the sequence was disregarded from further comparison. The four retrieved sequences were placed into a multiple sequence alignment using CLC Genomics Workbench 9.5.4 (https://www.qiagenbioinformatics.com/) under default parameters. Protein models were constructed from the amino acid sequences using the PHYRE2 web service (Kelley et al., 2015) under the 'intensive' modelling option. Structural models were visualised using The PyMOL Molecular Graphics System, Version 2.0.4 Schrödinger, LLC.

2.2. Study site and animals

A total of 158 Boer goats of both sexes, aged 3 to 5 months, were sampled from a goat farm in Lancaster, England, three times at four week intervals from August to October 2014. All sampling from animals was carried out after review by the local Animal Ethics Committee of the University of Glasgow and the UK Home Office. Information collected on each goat included date of birth, type of birth (singleton, twin or triplet), sex, father, mother and grandparents. The goats were allowed to graze on the pasture throughout summer, provided water *ad libitum* and given a supplement of commercial pelleted feed. All goats were drenched with 2.5% benzimidazole (7.5 mg/kg body weight) in August and 0.1% moxidectin (0.2 mg/kg body weight) in September and October according to the manufacturer's recommended dosages. Blood and faecal samples were collected one week or less before drenching. The body weight of each goat was taken prior to sampling. Faecal samples were collected from the rectum and blood samples were collected from the jugular vein from each goat in the morning. The samples were transported on the same day of sampling to School of Veterinary Medicine, University of Glasgow where laboratory analysis was carried out. The faecal samples were used to determine the FEC. Pooled faecal samples collected in each month were cultured to harvest the infective stage larvae (L3) for identification to genus level based on the instruction provided by Ministry of Agriculture, Fisheries and Food of Great Britain (1986). The blood samples were used to measure peripheral eosinophil counts, packed cell volume (PCV) and IgA activity.

2.3. Modified McMaster technique

The original McMaster technique to count nematode eggs in faeces (Gordon and Whitlock, 1939) was modified; briefly 3 *g* faeces were homogenized in 42 ml of water then filtered through a fine mesh sieve (aperture 250 µm). The filtrate was then centrifuged at 700 relative centrifugal force (rcf) for 5 min. The supernatant was discarded. The pellet was resuspended in 15 ml saturated sodium chloride solution (SG = 1.2) and used to fill four McMaster chambers. The number of eggs per gram (epg) of faeces was obtained by multiplying the total number of eggs counted by 25.

2.4. Faecal culture for identification and enumeration of L3

The faeces were broken up finely using either mortar and pestle or gloved hand depending on the faecal consistency to give a crumbly mixture. The faecal mixture of 10 to 20 g was then packed in a container and moistened with distilled water. A moistened filter paper was used to cover the packed faeces and checked daily for moisture content. Distilled water was sprinkled on the filter paper when it appeared dry. The faecal culture was incubated at room temperature for 14 to 16 days. On the final day of incubation, the filter paper was removed. Next, the container was filled to the brim with lukewarm distilled water. The container was then inverted onto a petri dish. Afterwards, the petri dish was filled with lukewarm distilled water and allowed to stand for 30 min for L3 migration from the faecal culture. Distilled water containing L3 in the petri dish was pipetted into a universal bottle and stored at 4 °C. A pipette was used to transfer 0.25 ml larval suspension into a cavity block. The L3 were killed by adding a drop of Lugol's iodine to the larval suspension. The killed L3 appeared to be straightened. Free living nematodes stained more yellowish whereas L3 remained less stained with Lugol's iodine. A drop of stained L3 suspension was placed on a microscope slide, covered with a coverslip and examined under 10x objective of a compound microscope. The L3 were enumerated and differentiated by observing the morphology of the head and sheath tail based on the manual provided by Ministry of Agriculture, Fisheries and Food of Great Britain (1986).

2.5. Peripheral eosinophil counts

Carpentiers eosinophil counting solution was prepared by adding 2 ml of 2% aqueous solution of Eosin Y, 3 ml of 40% formaldehyde saturated with calcium carbonate and 95 ml of distilled water. A 10 µl sample of blood was added to 90 µl Carpentiers solution and the eosinophils were counted in a haemocytometer. The preserved blood was stored at room temperature. Each cell counted represented 5.6 cells/µl of whole blood (Stear et al., 2002).

2.6. Packed cell volume

Blood collected in EDTA tube was taken by capillary action into a glass capillary tube. The unfilled end of the tube was sealed with Cristaseal (Hawksley and Sons Ltd). The PCV was determined by centrifuging the blood in a capillary tube at 220 rcf for five min and read with a rotoreader.

2.7. Elisa

A simple indirect ELISA was used to measure antibody activity against *T. circumcincta* L3. The L3 of *T. circumcincta* was a gift from D. Bartley (Moredun Research Institute) and was passaged in worm-free lambs reared at Cochno Farm, Glasgow. The L3 from faecal culture were harvested as the source of parasite antigen for the ELISA assay. Preparation of antigen from L3 followed the procedure described for L4 by Strain et al. (2002). The ELISA also followed the protocol described by Strain et al. (2002) except that the second antibody was a goat anti-mouse immunoglobulin conjugated to horse radish peroxidase and the optical density (OD) was read at 450 nm. A high standard was prepared by pooling plasma samples from different kids which had given high OD

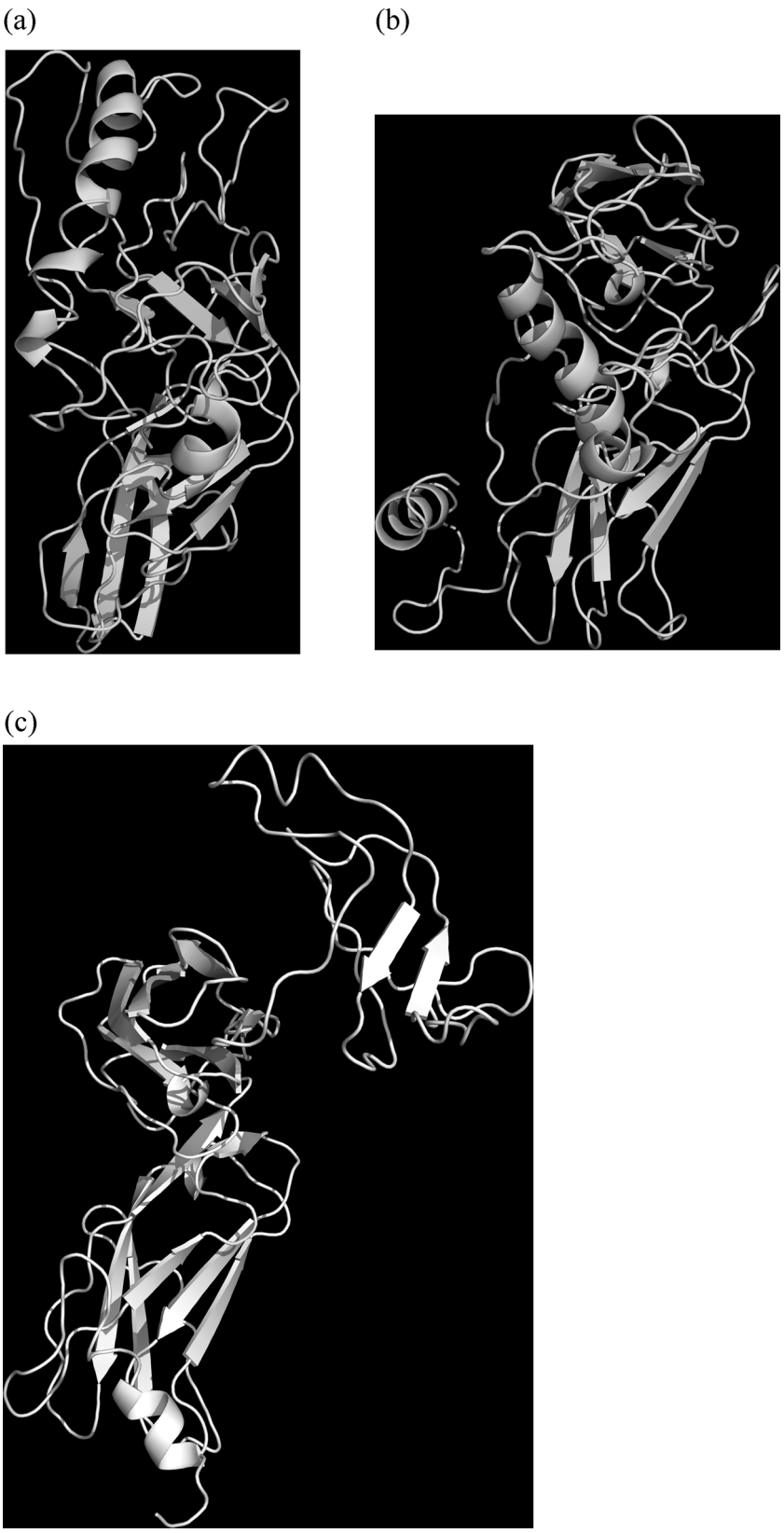

Fig. 1. Model of the high affinity IgA receptor in (a) humans (*Homo sapiens*), (b) sheep (*Ovis aries*) and (c) goats (*Capra hircus*). The goat receptor lacks the alpha helix which forms part of the transmembrane domain in human and sheep molecules.

in the preliminary studies. In this study, foetal bovine serum was used as the negative control. The positive and negative controls were also run in triplicate. The IgA OD index for each sample was determined by the following formula:

IgA OD index = (Sample mean – Negative control mean) / (Positive control mean – Negative control mean)

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CHFcaRI      1 MASRDVTLFCLVLCLGQKIQAQDGNFPIPIISATPSSVIPWNGSVKILCRGTLESYLYQL
OAFcaRI      1 MAPRDITLFCLVLCLGQKIQAQDGNFPIPIISATPSSVIPWNGSVKILCRGTLESYLYQL
HSFcaRI      1 MDPKQTTLLCLVLCLGQRIQAQEGDFPMPFISAKSSPVIPLDGSVKIQCQAIREAYLTQL
                  *  *

CHFcaRI     61 EILENLTYKQVEKQLGF--QEVAEFVINPVDTNTAGCYQCRYRREHHWSAPSEALELVVT
OAFcaRI     61 EILENLTYKQVEKQLGF--QEVAEFVINPMDTNTAGRYQCRYRRERHWSAPSEALELVVT
HSFcaRI     61 MIIKNSTYREIGRRLKFWNETDPEFVIDHMDANKAGRYQCQYRIGHYRFRYSDTLELVVT
                                            *       *       *

CHFcaRI    119 GLYDKPFLSTNGGHVAMPGENISFQCSSAHMSFDRFSLSRPGGPTLSRHRDARLQVDFTL
OAFcaRI    119 GLYDKPFLSTNGGHVAMPGENISFQCSSAHMSFDRFSLSRPGGPTLSRHRDVRLQVDFTL
HSFcaRI    121 GLYGKPFLSADRGLVLMPGENISLTCSSAHIPFDRFSLAKEGELSLPQHQSGEHPANFSL
                                                                      *

CHFcaRI    179 GPVNLSFSGVYTCYSWHSGRPYVWSAPSDALELVVTDTARQDHTTENWVRMGVAGLVLLA
OAFcaRI    179 GPVNLSFSGVYTCYGWHSGRPYLWSAPSDALELVVTDTASQDHTTENWVRMGVAGLVLLA
HSFcaRI    181 GPVDLNVSGIYRCYGWYNRSPYLWSFPSNALELVVTDSIHQDYTTQNLIRMAVAGLVLVA
                               *       *               *

CHFcaRI    239 LLAILAENRLGPQLPHQEDQQDLPDLSWSWQKSQTEWTFGLTPKDHQGDSWS
OAFcaRI    239 LLAILAENRLGPQLPHQEDQQDLPDLSRSWQKSRTEWTFGLTPKDHQKDSWS
HSFcaRI    241 LLAILVENWHSHTALNKEASADVAEPSWSQQMCQPGLTFARTPS-----VCK
                                          *     *             *
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Fig. 2. Multiple sequence alignment of the translated sequences for the human, sheep and goat high affinity IgA receptors. The binding domain (EC1) (Morton, Pleass, Woof, & Brandtzaeg, 2004) is labelled in **Bold**, the trans-membrane domain (van Dijk et al., 1996) is underlined and polymorphisms between CHFcaRI (goat) and OAFcaRI (sheep) are marked with an asterisk (*).

2.8. Statistical analysis

SAS 9.4 and SAS University edition software were used for statistical analyses. The UNIVARIATE procedure was used to determine means, standard deviations, minimum and maximum values. The UNIVARIATE procedure was also used to estimate the fit to continuous distributions such as the normal and gamma distributions. The Kolmogorov-Smirnov, Cramer-von Mises, and Anderson-Darling tests were employed for continuous distributions while the Shapiro-Wilk statistic was also used for testing the fit to a normal distribution. The distributions were plotted with the sgplot procedure. The negative binomial distribution has often been used to describe the distribution of FEC (Stear et al., 2008). The distribution has two parameters; the mean and a shape parameter k. The two parameters were estimated by maximum likelihood with the GENMOD procedure of SAS. Correlation analysis was determined using Spearman's Rank Correlation with the CORR procedure.

The SAS procedure GLIMMIX was used for generalised linear mixed modelling and repeated measures analysis. The repeated measures data were analysed by mixed model methods which are based on generalised least squares and maximum likelihood. In matrix notation the model is

$$\boldsymbol{Y} = \boldsymbol{X\beta} + \boldsymbol{e}$$

where **Y** is the vector of measurements, **X** is a matrix of known constants, $\boldsymbol{\beta}$ is a vector of fixed but unknown parameters while **e** is a vector of random errors.

In the first repeated measures analysis, the model assumed a negative binomial distribution for FEC and used a log link. Initially the full model included FEC, month of sampling, eosinophil count, IgA activity, type of birth and gender and all their interactions. However, this model did not converge despite specifying the value of the covariance parameters and increasing the convergence criterion from the default of 1×10^{-8}. Consequently, sex and type of birth were dropped from the model. Of the 158 animals sampled only 6 were male. Only 41 of the 158 goats were twin births and one animal was from a triple birth. In this reduced model, the interactions between month of sampling, IgA activity and eosinophilia were not significant. The final model examined the effects of month of sampling, IgA activity and eosinophilia on the number of eggs counted (epg/25). Five different covariance structures were compared: the models were compound symmetry, a first order autoregressive model, a Toeplitz model, a model that combined compound symmetry with first order autoregression and an unstructured covariance model. The pseudo-Bayes information criteria (BIC) were 1288.4, 1289.1, 1288.29, 1425.3 and 1274.6 respectively. The unstructured covariance model had the lowest value for the pseudo-BIC criterion and consequently, this was the most appropriate model for these data.

A repeated measures model was also used to examine the effects of parasitism on body weight. The glimmix procedure was used and the coding assumed that the residuals in the model followed a normal distribution. As both FEC and PCV were correlated with body weight on at least three occasions, a parasite index was created by adding together standardised PCV and FEC. The variables were standardised by subtracting the mean from each observation and dividing by the standard deviation. Five models were examined: compound symmetry, a first order autoregressive model, a Toeplitz model, a model that combined compound symmetry with first order autoregression and an unstructured covariance model. The values for Bayes Information criterion were 1663.4, 1664.4, 1668.2, 1667.6 and 1656.3 respectively. Again the unstructured model had the lowest BIC.

The final repeated measures model fitted the effect of the eosinophil and IgA responses on body weight. The analysis modelled body weight on month of sampling, eosinophilia and IgA activity and their interactions. A comparison of covariance structures indicated that compound symmetry provided the most appropriate structure. The BIC for the unstructured, compound symmetry, first order autoregression, Toeplitz and combined compound symmetry and first order autoregression were 1683.4, 1678.2, 1684.9, 1683.8 and 1683.8, respectively.

3. Results

Structural modelling of the high affinity IgA receptor in sheep, goats and humans showed that the sheep and human receptors had similar structures while the goat receptor had a different conformation (Fig. 1). In particular, the C terminus of the goat receptor was bent away from the main body of the protein and lacked the alpha helix (approximately 20 amino acids) within the trans-membrane domain. Multiple sequence alignment showed that *O. aries* and *C. hircus* sequences were highly similar (96% identity), with only 12 amino acid changes (Fig. 2). The *O. aries* and *C. hircus* proteins were dissimilar to the *H. sapiens* protein with only 55% and 54% identity respectively. Three amino acid were different in the binding domain (EC1) but there were no changes in the transmembrane domain. The loss of secondary structure in the transmembrane domain is therefore a consequence of sequence differences outside this domain.

Table 1 shows the species composition of the larvae recovered from faecal cultures. *T. circumcincta* was the dominant species in August,

Table 1
Percentage recovery of third stage larvae by species from faecal culture of Boer goats in August, September and October 2014.

Species	August	September	October
Teladorsagia circumcincta	93	90	92
Trichostrongylus spp.	7	10	8

Table 2
Summary statistics of Boer goats in August, September and October 2014.

Variable (unit)	Mean	Standard Deviation	Minimum	Maximum
FEC August (epg)	213	332	0	2025
FEC September (epg)	3240	3124	0	13850
FEC October (epg)	177	409	0	3300
PCV August (%)	26.5	4.1	13.5	37.0
PCV September (%)	24.6	3.5	13.5	34.0
PCV October (%)	25.3	3.5	12.5	33.0
IgA activity August (Anti-L3 OD)	0.68	0.25	0.09	1.13
IgA activity September (Anti-L3 OD)	0.69	0.23	0.08	1.23
IgA activity October (Anti-L3 OD)	0.50	0.22	0.04	1.15
Eosinophilia August (Cells / ul blood)	1542	689	0	3175
Eosinophilia September (Cells/µl blood)	1798	910	118	4917
Eosinophilia October (Cells/µl blood)	1303	904	6	3797
Body weight August (kg)	16.0	4.1	7.5	29.5
Body weight September (kg)	17.8	4.7	8.5	35.0
Body weight October (kg)	20.0	4.1	11.0	34.0

FEC = faecal egg counts; epg = eggs per gram; PCV = packed cell volume; IgA = Immunoglobulin A; OD = optical density index; BWT = body weight; kg = kilogram.

September and October with 90% or greater of the recovered larvae belonging to this species. The remaining species were all members of the genus Trichostrongylus.

Table 2 shows the mean, standard deviation and minimum and maximum values for FEC in the three months of sampling. The mean values were relatively low in August and October (about 200 epg) but the mean was 15 times higher in September. The standard deviations were similar to the mean values. As the standard deviation is the square root of the variance, the variances were clearly greater than the means at each time point, indicating overdispersion which is usual for FEC.

Table 2 also presents means, standard deviations and minimum and maximum values for PCV, IgA activity against L3, peripheral eosinophilia and body weight on the same three occasions. There was a slight drop in the PCV from August (26.5%) to September (24.6%) and October (25.3%). IgA activity was similar in August and September with OD indices of 0.68 and 0.69 but had declined in October to an OD index of 0.50. Peripheral eosinophilia was 1542 cells per µl blood in August, rose to nearly 1800 cells per µl in September and declined to only 1300 cells per µl in October. In contrast to the fluctuations in IgA activity and peripheral eosinophilia, body weight rose steadily with a mean value of 16 kg in August, nearly 18 kg in September and 20 kg in October.

The distribution of parasitological variables can inform the distribution of pathological effects in the flock and guide the statistical analysis. All five variables (FEC, PCV, IgA activity, eosinophilia and body weight) were plotted in each of the three months. Fig. 3a-e show the distribution among animals in September while Supplementary Fig. 1 shows the distributions in August and October.

FEC were low in August, high in September and low in October. FEC were right skewed and overdispersed in all three months. The default distribution for counts is often the Poisson distribution where the mean is equal to the variance. When the mean is greater than the variance, the negative binomial is commonly used as the default distribution. The negative binomial has several different parameterizations. In SAS the distribution can be described by two parameters: the mean (μ) and an index (k) of overdispersion where the variance = $\mu + k\mu^2$. The parameter k was estimated by maximum likelihood with the genmod procedure. In August, September and October, k was estimated as 4.96, 3.61 and 58.46 respectively. In all three months, k was significantly different from zero indicating overdispersion and that the Poisson distribution is not a good description of the data.

PCV were unimodal and approximately symmetrical in all three months. Shapiro–Wilk tests indicated that the distributions were not significantly different from a normal distribution in August ($p = 0.122$), September ($p = 0.748$) and October ($p = 0.063$).

The distributions of IgA activity and peripheral eosinophilia both showed a similar trend. They were slightly left skewed in August and September (Shapiro–Wilk tests for normality of eosinophilia in August $p = 0.329$ and September $p = 0.011$; of IgA activity in August $p = 0.001$ and September $p = 0.455$). In October, both peripheral eosinophilia and IgA activity were right skewed and more closely resembled a gamma distribution.

Body weight was unimodal but there was an excess of individuals with relatively high body weight compared to a normal distribution. (In August Shapiro–Wilk $p = 0.067$; In September Shapiro–Wilk $p = 0.003$; In October Shapiro–Wilk $p = 0.019$).

Table 3 shows the Spearman's rank correlations among FEC, PCV, IgA activity, peripheral eosinophilia and body weight in August, September and October.

Of the traits measured, body weight had the highest correlations between adjacent months at 0.83 and 0.91 ($p < 0.001$). PCV and IgA activity had moderate but significant correlations ($p > 0.05$). For PCV the correlations among adjacent months were 0.40 and 0.28. For IgA activity, the correlations were 0.46 and 0.46. The lowest correlations were among eosinophil counts and among FEC, which were not significant ($p > 0.05$). The correlations between adjacent months were 0.05 and −0.03 for eosinophilia and −0.08 and 0.09 for FEC.

There were very few significant correlations among the different variables (Table 3). Higher FEC in August, but not September and October, were associated with lower body weight. Similarly, in 8 of 9 comparisons, lower PCV were associated with low body weight. Neither IgA activity nor peripheral eosinophilia were associated with FEC. This suggests that IgA and eosinophils were not regulating nematode egg output. Only 1 of 9 comparisons between IgA and eosinophilia was significant; there was a weak negative correlation between eosinophil counts and IgA activity in August. Increased IgA activity in August, but not September and October, was associated with decreased body weight. Similarly, eosinophilia in August and October was associated with decreased body weight in September and October. Together these results suggest that IgA and eosinophilia have little effect on nematode egg production but that the cost of mounting these responses has a weak effect on growth of the goats or that animals with stronger responses had higher intensities of infection.

Non-parametric correlations are statistically robust but not very powerful. Therefore, a more powerful parametric repeated measures analysis was used to analyse the effect of the immune response on faecal egg count and on growth.

In the first repeated measures analysis, the model examined the effects of month of sampling, IgA activity and eosinophilia on the number of eggs counted (epg/25). There were significant differences among months ($p < 0.001$) but eosinophilia ($p = 0.418$) and IgA

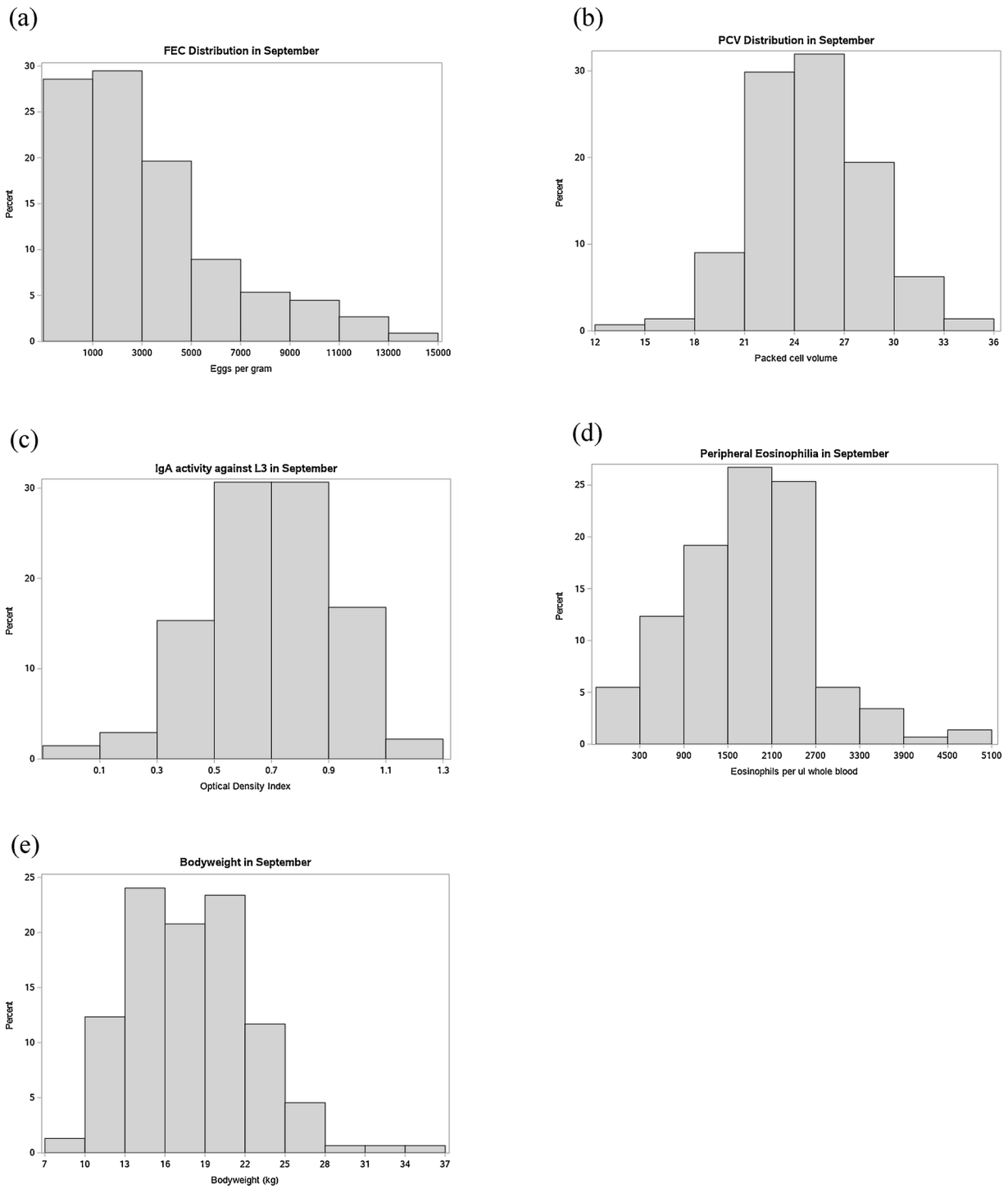

Fig. 3. Distribution in September among naturally infected Boer goats of (a) Faecal egg count (b) Packed cell volume, (c) IgA activity, (d) eosinophilia and (e) body weight.

activity ($p = 0.084$) were not significant. However, the estimated effects for IgA activity were significant in the three models ($0.01 < p < 0.05$) with similar pseudo-BIC criteria. In all three cases the effect of IgA activity was positive; increased FEC were associated with increased IgA activity.

A problem in assessing multiple immune responses simultaneously is collinearity. Only 1 of 9 correlations between IgA activity and peripheral eosinophilia was statistically significant but it is possible that both variables were independently correlated with the 'true' protective immune response against gastrointestinal nematodes. We therefore created a standardised immune index. IgA activity and peripheral eosinophilia were first standardised by subtracting the mean from each observation and dividing by the standard deviation. These standardised variables were added together to create an immune index. This immune index was then fitted along with month of sampling and their interaction. The interaction was not significant and it was dropped from the final model. In the final model, the FEC differed among months ($p < 0.001$) but the immune index was not associated with differences in faecal egg count ($p = 0.512$).

A repeated measures model was also used to examine the effects of

Table 3
Rank Correlations among FEC, PCV, IgA, EO and BWT in August, September and October.

	FECA	FEC S	FECO	PCVA	PCVS	PCVO	IgAA	IgAS	IgAO	EOA	EO S	EOO	BWTA	BWTS
FECS	−.08													
FECO	−.08	.09												
PCVA	−.17	.20	.02											
PCVS	−.17	.04	.04	**.46****										
PCVO	−.05	−.16	.08	**.27****	**.28****									
IgAA	−.03	.16	.07	.01	−.09	−.08								
IgAS	−.04	.05	.16	.08	.00	−.02	**.46****							
IgAO	−.07	.08	.09	.13	.01	−.07	**.40****	**.46****						
EOA	.11	−.05	−.07	.09	.00	−.15	**−.18***	−.08	−.04					
EOS	−.17	−.08	−.09	.13	.11	−.01	.03	.01	.01	.05				
EOO	.11	−.05	.08	−.02	−.16	−.16	.10	−.08	−.07	.03	−.03			
BWTA	**−.34****	.10	.01	**.25****	**.37****	.08	**−.21****	−.07	−.07	.00	.10	−.10		
BWTS	**−.36****	−.04	.02	**.26****	**.41****	**.21****	**−.26****	−.03	−.09	**−.18***	.01	**−.16***	**.83****	
BWTO	**−.33****	.00	−.02	**.29****	**.39****	**.18***	**−.25****	−.05	−.06	**−.13***	.06	**−.23****	**.88****	**.91****

FEC = faecal egg counts; PCV = packed cell volume; IgA = Immunoglobulin A optical density index; EO = peripheral eosinophil counts; BWT = body weight; A = August; S = September; O = October; * = $p < 0.05$; ** = $p < 0.01$.

parasitism on body weight. The glimmix procedure was used and the coding assumed that the residuals in the model followed a normal distribution. As both faecal egg count and PCV were correlated with body weight on at least three occasions, a parasite index was created by adding together standardised PCV and faecal egg count. As before, the variables were standardised by subtracting the mean from each observation and dividing by the standard deviation. In this model the month of sampling was associated with differences in body weight ($p < 0.001$) but not the parasite index ($p = 0.204$). A similar repeated measures analysis for body weight fitted just month of sampling, faecal egg count and their interaction. The effect of month was significant ($p < 0.001$) but not the effect of faecal egg count ($p = 0.181$) or the interaction between month and faecal egg count ($p = 0.107$).

The final repeated measures analysis modelled body weight on month of sampling, eosinophilia and IgA activity and their interactions. There were significant effects of month of sampling ($p < 0.001$), the interaction of month with eosinophilia ($p < 0.001$), the interaction of month with IgA activity ($p = 0.035$) and the three way interaction with month, eosinophilia and IgA activity ($p = 0.035$). The main effects of eosinophilia ($p = 0.244$) and IgA activity ($p = 0.233$) as well as their interaction ($p = 0.503$) were not significant. These results reinforce the correlation analysis in showing that the eosinophil and IgA responses to parasite infection did affect body weight but only in certain months. Fitting the immune index, month and their interaction in the compound symmetry model gave a similar result; there was a significant effect on body weight of month ($p < 0.001$) and the interaction between month and the immune index ($p = 0.001$) but no direct effect of the immune index ($p = 0.244$).

4. Discussion

This study has used a combination of bioinformatic and statistical analyses to examine the IgA and eosinophil response against gastrointestinal nematodes during natural infection of goats. Molecular modelling suggested the hypothesis that the goat IgA receptor on eosinophils may, like the mouse IgA receptor, be dysfunctional. However, further study will be necessary to test this hypothesis. FEC, PCV, IgA activity against L3, peripheral eosinophilia and body weight were measured in a flock of 158 goats in August, September and October. Therefore, it was appropriate to examine the distributions of the measured variables in detail in order to determine the best way to summarise the data and the most appropriate analyses. The distribution of faecal egg count was overdispersed and the negative binomial was used to summarise the data. Another method that has been used to summarise egg count data is Taylor's power law (Boag et al., 1992; Stear et al., 1998).

However, this relationship requires several populations to estimate the relationship between the mean and the variance. Our three populations (August, September and October) are insufficient to provide reliable estimates of the relationship between population means and variances.

PCV were similar to a normal distribution. This was not unexpected as PCV often conform to a normal distribution in other species, including humans (Marx and Vergouwen, 1998). Both immune variables, eosinophilia and IgA activity, were similar to a normal distribution in August and September but became right-skewed in October and more similar to a gamma distribution. Body weight almost but not quite followed a normal distribution. In practice, observations seldom conform exactly to a specified distribution and there is an art to deciding between powerful parametric analyses which assume specific distributions and less powerful but more robust distribution-free statistical procedures. Here, both powerful parametric procedures were employed such as generalised linear modelling and the distribution-free Spearman's correlation to interpret the relationships between parasitism, the immune response and growth in goats.

The correlations among the same variable measured at different times can indicate the appropriate covariance structure for statistical analysis. Correlations can also indicate the robustness of the procedures and provide a guide for the strength of genetic control because the product-moment correlation between two variables (also known as the repeatability) must exceed the heritability. The correlations were high for body weight, moderate for PCV and for L3-specific IgA activity but very low for eosinophilia and faecal egg count. In comparison the correlations for FEC in lambs at 4 to 6 month of age are usually higher and above 0.2 (Bishop et al., 1996). Similarly, the correlations among IgA activity in lambs at 4 to 6 month of age were higher; they were 0.55 between August and September and 0.50 between September and October (Strain et al., 2002). In the same lambs, the correlation among eosinophil counts in August and September was 0.4 (Stear et al., 2002).

The associations of IgA activity and eosinophilia with faecal egg count were also weaker in goats than sheep. Strong IgA and eosinophil responses in 4 to 6 month old lambs are associated with decreased FEC (Stear et al., 2002; Strain et al., 2002) but this is not the case in goats (McBean et al., 2016; Paterson et al., 1996a, b). There were associations at certain times but these were positive associations; strong IgA responses were associated with increased egg counts. Positive associations suggest that high intensities of infection may be driving high responses. These strong immune responses were detrimental to growth of the goat kids but only at certain times. It is not obvious why the immune response should have had a stronger association with body weight in August than subsequent months. However, the result appears robust as it was found in both the non-parametric correlations and in

the repeated measures analysis.

The weak and inconsistent associations between faecal egg count and body weight may be a consequence of regular and effective anthelmintic treatment. Previous research has indicated that exposure to nematode infection reduces growth rate (Coop et al., 1985). However, the pathology may be predominantly mediated by the immune system rather than directly by the parasite (Greer, 2008; Stear et al., 2003).

In conclusion, the absence of associations between increased parasite-specific IgA, peripheral eosinophilia and faecal egg count supports the idea that these components of the protective immune response in sheep are ineffective in goats. Molecular modelling suggests that the defect may lie in the IgA receptor on eosinophils.

Declarations of interest

There is no declaration of interest.

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Acknowledgements

This study was supported by the Ministry of Higher Education Malaysia and Universiti Malaysia Kelantan in the forms of scholarship, bench fees and emoluments but did not have any additional role in the study design, data collection and analysis, report writing, decision to publish, or preparation of the manuscript. We would also like to acknowledge the Moredun Institute, Edinburgh, Scotland for the *T. circumcincta* L3 used in this study.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:10.1016/j.vetpar0.2018.10.014.

References

Amarante, A.F.T., Bricarello, P.A., Huntley, J.F., 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.

Bishop, S., Bairden, K., McKellar, Q., Park, M., Stear, M., 1996. Genetic parameters for faecal egg count following mixed, natural, predominantly *Ostertagia circumcincta* infection and relationships with live weight in young lambs. Anim. Sci. 63, 423–428.

Boag, B., Hackett, C.A., Topham, P.B., 1992. The use of Taylor's power law to describe the aggregated distribution of gastro-intestinal nematodes of sheep. Int. J. Parasitol. 22, 267–270.

Coop, R.L., Graham, R.B., Jackson, F., Wright, S.E., Angus, K.W., 1985. Effect of experimental *Ostertagia circumcincta* infection on the performance of grazing lambs. Res. Vet. Sci. 38, 282–287.

de la Chevrotière, C., Bambou, J.C., Arquet, R., Jacquiet, P., Mandonnet, N., 2012. Genetic analysis of the potential role of IgA and IgE responses against *Haemonchus contortus* in parasite resistance of Creole goats. Vet. Parasitol. 186, 337–343.

Dunsmore, J.D., 1961. Effect of whole-body irradiation and cortisone on the development of *Ostertagia* species in sheep. Nature 192, 139–140.

Gordon, H.M., Whitlock, H.V., 1939. A new technique for counting nematode eggs in sheep faeces. J. C. Sci. Ind. Res, Australia 12, 50–52.

Greer, A.W., 2008. Trade-offs and benefits: implications of promoting a strong immunity to gastrointestinal parasites of sheep. Parasite Immunol. 30, 123–132.

Halliday, A.M., McAllister, H.C., Smith, W.D., 2010. Kinetics of the local immune response in the gastric lymph of lambs after primary and challenge infection with *Teladorsagia circumcincta*. Parasite Immunol. 32, 81–90.

Henderson, N.G., Stear, M.J., 2006. Eosinophil and IgA responses in sheep infected with *Teladorsagia circumcincta*. Vet. Immunol. Immunopathol. 112, 62–66.

Hoste, H., Sotiraki, S., Landau, S.Y., Jackson, F., Beveridge, I., 2010. Goat-nematode interactions: think differently. Trends Parasitol. 26, 376–381.

Hoste, H., Torres-Acosta, J.F.J., Aguilar-Cabellero, A.J., 2008. Nutrition-parasite interactions in goats: is immunoregulation involved in the control of gastrointestinal nematodes? Parasite Immunol. 30, 79–88.

Huntley, J.F., Patterson, M., Mackellar, A., Jackson, F., Stevenson, L.M., Coop, R.L., 1995. A comparison of the mast cell and eosinophil responses of sheep and goats to gastrointestinal nematode infections. Res. Vet. Sci. 58, 5–10.

Kelley, L.A., Mezulis, S., Yates, C.M., Wass, M.N., Sternberg, M.J.E., 2015. The Phyre2 web portal for protein modeling, prediction and analysis. Nat. Protoc. 10, 845–858.

Le Jambre, L.F., Royal, W.M., 1976. A comparison of worm burdens in grazing merino sheep and angora goats. Aust. Vet. J. 52, 181–183.

Mandonnet, N., Aumont, G., Fluery, J., Arquet, R., Varo, H., Gruner, L., Bouix, J., Tien, Vu, Khang, J., 2001. Assessment of genetic variability of resistance to gastrointestinal nematode parasites in Creole goats in the humid tropics. J. Anim. Sci. 79, 1706–1712.

Marx, J., Vergouwen, P., 1998. Packed-cell volume in elite athletes. Lancet 352, 451.

McBean, D., Nath, M., Kenyon, F., Zile, K., Bartley, D.J., Jackson, F., 2016. Faecal egg counts and immune markers in a line of Scottish Cashmere goats selected for resistance to gastrointestinal nematode parasite infection. Vet. Parasitol. 229, 1–8.

McRae, K.M., Stear, M.J., Good, B., Keane, O.M., 2015. The host immune response to gastrointestinal nematode infection in sheep. Parasite Immunol. 37, 605–613.

Miller, H.R.P., 1984. The protective mucosal immune response against gastrointestinal nematodes in ruminants and laboratory animals. Vet. Immunol. Immunopathol. 6, 167–259.

Ministry of Agriculture, Fisheries and Food of Great Britain. (1986). Manual of veterinary parasitological laboratory techniques 418. HM Stationery Office.

Murphy, L., Eckersall, P.D., Bishop, S.C., Pettit, J.J., Huntley, J.F., Burchmore, R., Stear, M.J., 2010. Genetic variation among lambs in peripheral IgE activity against the larval stages of *Teladorsagia circumcincta*. Parasitology 137, 1249–1260.

Paterson, D.M., Jackson, F., Huntley, J.F., Stevenson, L.M., Jones, D.G., Jackson, E., Russel, A.J.F., 1996a. The response of breeding does to nematodiasis: segregation into "responders" and "non-responders". Int. J. Parasitol. 26, 1295–1303.

Paterson, D.M., Jackson, F., Huntley, J.F., Stevenson, L.M., Jones, D.G., Jackson, E., Russel, A.J.F., 1996b. Studies on caprine responsiveness to nematodiasis: segregation of male goats into responders and non-responders. Int. J. Parasitol. 26, 187–194.

Seaton, D.S., Jackson, F., Smith, W.D., Angus, K.W., 1989. Development of immunity to incoming radiolabelled larvae in lambs continuously infected with *Ostertagia circumcincta*. Res. Vet. Sci. 46, 241–246.

Smith, W.D., Jackson, F., Jackson, E., Williams, J., 1985. Age immunity to *Ostertagia circumcincta*: comparison of the local immune responses of 4 1/2 and 10 month-old lambs. J. Comp. Pathol. 95, 235–245.

Smith, W.D., Jackson, F., Jackson, E., Williams, J., Miller, H.R.P., 1984. Manifestations of resistance to ovine ostertagiasis associated with immunological responses in the gastric lymph. J. Comp. Pathol. 94, 591–601.

Stear, M.J., Bairden, K., Bishop, S.C., Gettinby, G., McKellar, Q.A., Park, M., Strain, S.A.J., Wallace, D.S., 1998. The processes influencing the distribution of parasitic nematodes among naturally infected lambs. Parasitology 117, 165–171.

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., Bishop, S.C., Henderson, N.G., Scott, I., 2003. A key mechanism of pathogenesis in sheep infected with the nematode *Teladorsagia circumcincta*. Anim. Health Res. Rev. 4, 45–52.

Stear, M.J., Fitton, L.A., Innocent, G.T., Murphy, L., Rennie, K., Matthews, L., 2008. The dynamic influence of genetic variation on the susceptibility of sheep to gastrointestinal nematode infection. J. R. Soc. Interface 4, 767–776.

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.

Urban, J.F., Katona, I.M., Paul, W.E., Finkelman, F.D., 1991. Interleukin 4 is important in protective immunity to a gastrointestinal nematode infection in mice. Proceedings of the National Academy (USA) 88, 5513–5517.

van Egmond, M., Damen, C.A., van Spriel, C.A., Vidarsson, G., van Garderen, E., van de Winkel, J.G.J., 2001. IgA and the IgA fc receptor. Trends Immunol. 22, 205–211.