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Kinetics of IgA and eosinophils following a low-dose, predominantly *Haemonchus contortus* infection of Boer goats.

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Structured abstract

Aims:

Most breeds of goat are more susceptible to nematode infection than sheep and this appears to be a consequence of less effective immune responses. Several papers have considered the effectiveness of eosinophils and Immunoglobulin A (IgA) in goats but differences in the induction of responses have not been studied in the same detail. The aim of this study was to look at the induction of eosinophil and IgA responses in Boer goats reared indoors under intensive conditions.

Methods and results:

The goats were experimentally infected with a low dose of 2400 *Haemonchus contortus, Trichostrongylus* spp. and *Oesophagostomum* spp. at a 6:1:1 ratio. Faecal egg counts (FEC), packed cell volume (PCV), IgA activity against third-stage larvae and peripheral eosinophilia were measured twice a week for eight weeks. The infection generated an IgA response but did not significantly increase peripheral eosinophilia in the 25 infected kids compared to the 4 control animals. FEC was not associated with IgA activity or eosinophilia.

Conclusion:

A detailed analysis of IgA and eosinophil responses to deliberate nematode infection in Boer goats showed that there was an increase in nematode-specific IgA activity but no detectable eosinophil response. In addition, there was no association between increased IgA activity or eosinophilia with egg counts and worm burdens. These suggest that IgA and eosinophils do not act to control nematode infection in goats.

Keywords:

Goat; nematode; *Haemonchus contortus;* IgA; eosinophil; kinetics; repeatability

Introduction

Nematode infection is one of the most severe diseases faced by small ruminants such as sheep and goats [1]. The two species have developed different ways to reduce the ravages of disease. Sheep mount strong immune responses while goats minimise exposure by browsing leaves rather than grazing grass [2]. In both sheep [3,4] and goats [5-7] some breeds mount more effective immune responses than others.

Three methods that are being explored to control nematode infections in small ruminants are the use of genetically resistant animals [8-11], dietary protein supplementation [12-16] and the development of vaccines [17,18]. These three methods depend at least partly upon enhancing the immune response to nematodes [19]. Therefore, characterizing the immune response in goats and identifying the reasons why many goats mount relatively weak responses compared to sheep could provide useful insights for nematode control.

In sheep, parasite-specific Immunoglobulin A (IgA) activity and eosinophilia are negatively associated with faecal egg counts [20-22]; i.e. high immune responses are associated with low faecal egg counts. In contrast, natural infections in Scottish Cashmere and Creole goats with predominantly *Teladorsagia circumcincta* and *Haemonchus contortus*, have not shown these negative associations [10,23] In addition, there was no negative association between FEC and parasite-specific IgA activity or eosinophil responses in Boer goats in a mixed natural infection dominated by *T. circumcincta* [24]. In the same study, molecular modelling suggested that the IgA receptor on eosinophils was dysfunctional.

In sheep there are two major immune responses: type I hypersensitivity responses are associated with reduced worm numbers [25,26] while increased nematode-specific IgA activity and eosinophilia are associated with reduced worm length and fecundity [21,27]. However, there has been little attention paid to differences between the species in the induction of immune responses.

In natural infections, sheep and goats are commonly infected with a mix of gastrointestinal nematode species. Although *H. contortus* infection generally occurs together with other gastrointestinal nematodes, it consistently dominates the infection among livestock in tropical or

subtropical countries[28]. For example, milk production in Saanen goats naturally infected with *H. contortus* and *Trichostrongylus colubriformis* and subsequently treated with anthelmintic is significantly higher (399.5 L ± 34.0 L; $p<0.05$) when compared to untreated goats (281.6 L ± 37.5 L) [29].

Sheep and goats are usually kept indoors in Southeast Asian countries where land and capital are scarce. In this intensive farming system, livestock are completely confined and entirely dependent on stall-feeding [30]. This approach limits exposure to L3 and reduces nematode infection among animals. Therefore, low levels of mixed infection may occur naturally in small ruminants that are managed intensively. Hence, this paper describes the induction of IgA and eosinophil responses in goats experimentally infected with a low dose of *H. contortus, Trichostrongylus* spp. and *Oesophagostomum* spp.

2. Materials and Methods

2.1 Study sites and animals

Thirty goats were selected randomly from a herd of 100 goats aged from 3 to 7 months old in a Boer goat farm in Selangor, Malaysia. All of the selected goats were females. The information collected on each goat included date of birth, type of birth (singleton or twin), sex, father, mother and grandparents. The goats were kept in wooden slatted-floor houses and provided with water *ad libitum* 24-hours a day. The goats were given fresh cut grasses, silage and a supplement of commercial pelleted feed. All goats were drenched with 2.5 % benzimidazole in February 2015 according to the manufacturer's recommended dosage (7.5 mg / kg body weight). The bodyweight of each goat was taken prior to sampling in April and May 2015. Faecal samples were collected per rectum and blood samples were collected via the jugular vein from each goat in the morning once every four weeks from April to June 2015. The faecal samples were subjected to a modified McMaster technique and the blood samples were used to measure IgA, PCV and peripheral eosinophil counts. All goats were confirmed to have zero FEC from April till June 2015.

The 30 goats were transferred into a different building and kept isolated in five pens. The goats were drenched with 2.7 % levamisole according to the manufacturer's recommended dosage (8.0 mg / kg body weight) to eliminate any infections. All goats had zero FEC four weeks after a single levamisole drenching. A monospecific culture of *H. contortus* that was passaged from donor sheep in the Veterinary Research Institute, Malaysia was combined with a mixed culture from the Parasitology Laboratory, Faculty of Veterinary Medicine, Universiti Putra Malaysia (UPM). The L3 stock from UPM was collected from naturally infected sheep and goats. The L3 used to infect the goats were kept at 4^{o}C for three to six months before the start of the experiment. Twenty-five goats were infected with 2400 L3 of *H. contortus, Trichostrongylus* spp. and *Oesophagostomum* spp. at a 6:1:1 ratio while the other remaining goats served as negative controls. One control animal was inadvertently infected and was dropped from the control group, leaving 25 infected and four control kids. At the start of the infection, the mean body weight of the 25 infected goats was 16.3 + 3.5 kg (mean ± s.d) while the mean body weight of the 4 control goats was 15.5 + 1.9 kg. Their mean ages were 283 + 29 and 308 + 3 days, respectively. The bodyweights and ages at infection were not significantly different in the infected and control groups ($p>0.05$). The goats were slaughtered in the farm after the 16th sampling (day 52 post-infection) to collect the gastrointestinal tracts. The goats were humanely slaughtered according to the halal slaughtering

procedure [31]. The research was conducted with similar procedures that were approved by Animal Ethics Committee of the University of Glasgow and the UK Home Office 24 (PPL 60/4077).

2.2 Modified McMaster technique

The original McMaster technique to count nematode eggs in faeces [32] was modified [24]. As each faecal sample had two replicates where four McMaster chambers were used to count the nematode eggs, the number of eggs per gram (epg) of faeces was obtained by multiplying the total number of eggs counted by 25. According to the manual provided by the Ministry of Agriculture, Fisheries and Food of Great Britain (1986), the consistency of the faeces will affect the epg, thus various corrections have been proposed to compensate for variation in the moisture content of the faeces. To estimate the epg for watery faeces, the number of eggs counted in four McMaster chambers was multiplied by 12.5 based on the standard procedure at the Veterinary Parasitology Laboratory, Faculty of Veterinary Medicine, Universiti Putra Malaysia.

2.3 Faecal culture

Preparation of faecal culture followed our previously published procedure [24] except that the incubation period at room temperature was seven days. Preparation of L3 for identification and enumeration also followed our previously published procedure [24]. The L3 were enumerated and identified by observing their head and sheath tail morphology based on the manual provided by Ministry of Agriculture, Fisheries and Food of Great Britain (1986).

2.5. Packed cell volume

Packed cell volume (PCV) was measured by filling glass capillary tubes with blood samples from EDTA tubes, sealed with Cristaseal (Hawksley and Sons Ltd), centrifuged at 220 rcf for five min and read with a rotoreader [24].

2.6. Peripheral eosinophil counts

The method to count peripheral eosinophils followed our previously published procedure [22] . Two ml of 2 % aqueous solution of Eosin Y, 3 ml of 40 % formaldehyde saturated with calcium carbonate and 95 ml of distilled water were added together to prepare Carpentiers eosinophil counting solution. A 10 µl blood sample was added to 90 µl Carpentiers solution and stored at

room temperature. The eosinophils were counted in a haemocytometer and each cell counted represented 5.6 cells/µl of whole blood.

2.7. ELISA

The pooled faeces of experimentally infected goats were cultured to obtain L3 stock as the source of parasite antigen for ELISA assay. The final ratio of *H. contortus*: *Trichostrongylus* sp: *Oesophagostomum* sp. was 14:2:1. L3 were pooled to prepare the parasite antigen from these three genera as described for fourth-stage larvae (L4) [20]. Plasma samples were used in an ELISA protocol following a previously described method [20] except that the concentration of primary and secondary antibody was based on the preliminary ELISA optimization at 1:100 and 1:2000 dilution in PBS-T respectively, the secondary antibody was a goat anti-mouse immunoglobulin conjugated to horse radish peroxidase and the optical density (OD) was read at 450 nm. Plasma samples from different kids which had given high OD in the preliminary studies were pooled to prepare the positive control while foetal bovine serum was used as the negative control. Both positive and negative controls were run in triplicate. The positive control also acts as a reference standard. The formula to determine the IgA OD index for each sample as follows:

$$\text{IgA OD Index} = \frac{(\text{Sample mean} - \text{Negative control mean})}{(\text{Positive control mean} - \text{Negative control mean})}$$

2.8 Necropsy and total worm counts

The necropsy technique and the procedure for estimating total worm counts were based on protocols in the Manual of Veterinary Parasitological Laboratory Techniques [33]. The abomasum from a slaughtered kid was ligated and opened along the greater curvature. The abomasal contents and washing were collected into a graduated bucket and made up to 1 L with tap water. After mixing, 100 ml were transferred into a glass jar with a sieve-like lid. The jar was inverted and vigorously shaken until most of the fluid contents were removed. This process was repeated until the contents were apparently clear with a final volume of approximately 100 ml. The final contents of the jar were then transferred into a 120 ml specimen bottle. The worms were preserved by adding 5 ml of 75 % ethanol into the specimen bottle.

The worms were identified, counted, sexed and measured under a stereo microscope at x15 magnification. Based on the dilution factor of abomasal contents, each nematode counted

represented 10 worms [34]. A similar process was used to count and identify worms in the small and large intestines.

2.9. Statistical analysis

SAS 9.4 and SAS University edition software were used for statistical analyses. The MEANS and UNIVARIATE procedures were used to determine means, standard deviations, minimum and maximum values. Regression analysis of correlations among times of sampling were assessed in generalised linear mixed models with the GLIMMIX procedure. Similarly, the random effect of animal and the fixed effect of infection group on FEC, PCV, IgA activity and eosinophilia were also assessed in generalised linear mixed models with the GLIMMIX procedure. Animal was nested within group (infected or not) and group was fitted separately as a fixed effect. Gamma distributions with log links were fitted for FEC, IgA activity and eosinophilia while a normal distribution was assumed for PCV. One way regressions and correlations were used to examine the relationship between worm number and worm length and the average IgA activity, peripheral eosinophil count, PCV and FEC over the course of the infection. The responses were plotted with the sgplot procedure.

The Glimmix procedure was also used to examine the relationships among FEC, PCV, IgA activity and peripheral eosinophilia. The decline in the repeatabilities over time suggested that autoregressive models of order 1 would be most suitable for the data. We compared five different covariance structures (unstructured, compound symmetry, autoregressive of order one (ar(1)), Toeplitz and a model with the random effect of animal superimposed on an autoregressive structure. Three models failed to converge and the autoregressive model had a lower pseudo-BIC than the compound symmetry model. The ar(1) model was used to test the association between FEC and PCV. The response variable was FEC and the explanatory variables were the interaction between group (infected or not) and day of sampling as well as the fixed effect of PCV. The association between FEC and the immune response was examined in separate models that fitted interaction between group (infected or not) and day of sampling as well as the fixed effects of IgA activity and eosinophilia. Finally the relationship between IgA activity and eosinophilia was examined in two separate models that fitted IgA activity or eosinophilia as the response variable, group-by-day interaction and eosinophilia or IgA activity as the two response variables. The

procedure generates pseudo-likelihoods and pseudo-BIC (Bayes information criterion) was used to decide among models. BIC is also known as SBC [35].

Correlations and Cronbach's alpha coefficient were determined using Spearman's Rank Correlation by the CORR procedure. Cronbach's alpha coefficient estimates the internal consistency of a test or the average correlation of items within the test [36]. Cronbach's alpha coefficients above 0.70 are considered acceptable [37].

The repeatability of traits following infection has been estimated only occasionally in parasitological investigations of livestock [38]. The repeatability provides a measure of quality control, it indicates the similarity between measurements taken at different times during the infection and provides guidelines for the optimal time of sampling and the optimal number of samples to identify relatively resistant and susceptible animals. The repeatability is particularly suitable for studying responses to infection because it is a ratio of variances and as such it is not affected by changes in the mean that occur in the response to infection. The repeatability is the proportion of the total phenotypic variance due to variation among animals [39]. It was estimated by fitting the effect of animal in a linear model with the mixed procedure in SAS.

Results

The L3 percentages of each species recovered from faecal culture from experimentally infected Boer goats was, as expected, similar to the composition of the infection mixture. The predominant nematode recovered from culture was *H. contortus* (82%). The two remaining taxa were *Trichostrongylus* spp. (12%) and *Oesophagostomum* spp. (6%).

Cronbach's alpha coefficient was used to assess the reliability of FEC, PCV, IgA activity and peripheral eosinophilia. Cronbach's standardized alpha coefficient for FEC was 0.78. All alpha coefficients obtained after dropping observations from days 21 to 52 were greater than 0.70. No eggs were seen before 21 days after infection. The standardized alpha coefficient for PCV was 0.91. All alpha coefficients obtained after dropping measurements made at single time points were between 0.89 and 0.92. For IgA, the standardized alpha coefficient was 0.97. All alpha coefficients obtained after dropping single time points were 0.97. The standardized alpha coefficient for peripheral eosinophilia was 0.78 and the values obtained from separately dropping the measurements made on each day fell between 0.75 and 0.78. The internal consistency of PCV and IgA was higher than FEC and peripheral eosinophilia but all tests gave acceptable reliabilities.

Fig. 1 shows the FEC and PCV response following experimental infection. FEC were zero until 21 days after infection. FEC then rose to a peak of 567 epg at 49 days post infection before dropping on day 52. The PCV fell following infection from an initial value of 30.5 to 22.1; 28 days after infection. PCV subsequently recovered (26.1) 52 days after infection. However, the reduction in PCV was not significantly different between the 25 infected kids compared to the 4 control animals ($p=0.850$).

Fig. 2 illustrates the kinetics of the IgA and eosinophil responses following experimental infection. IgA activity is expressed as a percentage of a reference standard. IgA activity peaked at day 17 following infection then dropped below the starting value. The values in the uninfected controls were below the values in the experimentally infected animals, except for a spike of IgA between days 30 and 40. This spike was due to a response in just one animal. It is possible that this individual was accidentally infected. We have not attempted statistical comparisons between control and infected kids. In contrast to IgA activity, peripheral eosinophilia remained relatively stable until the 28th day after infection then dropped. At the end of the experiment, peripheral

eosinophilia was approximately half the starting value. As expected, infection resulted in increased nematode-specific IgA activity ($p<0.001$). However, the infection did not significantly increase peripheral eosinophilia in the 25 infected kids compared to the 4 control animals ($p=0.233$).

Generalised linear mixed modelling showed that there were significant differences among animals following infection ($p<0.001$ for FEC, PCV, IgA activity and eosinophilia). FEC varied among animals from 0 to 275 on day 21 at the end of the prepatent period and from 0 to 3500 epg on day 49 at the time of the peak response. PCV varied from 21.5 to 37.5 on day 0, from 18.0 to 27.5 on day 28 at the lowest point and from 24.5 to 29.5 on day 52. Parasite-specific IgA activity expressed as a proportion of a high standard varied from 0.11 to 1.47 on day 0, and from 0.18 to 3.00 at the time of the peak response on day 17. Eosinophilia varied from 0 to 425.6 eosinophils per ul blood on day 0, 16.8 to 364.0 eosinophils per ul blood on day 28 and 0 to 324.8 eosinophils per ul blood on day 52.

The repeatability of parasitological measurements such as FEC and PCV depends upon the interval between collection of samples (Fig. 3). For FEC, samples taken before day 21 had zero epg and did not contribute to the repeatability of FEC. Among the remaining FEC samples, the mean repeatability (r) of samples taken at three day intervals was 0.33 (n=5); for samples taken at 4 day intervals r=0.27 (n=4), for samples taken at 7 day intervals, r=0.28 (n=8); for samples taken at 14 day intervals r=0.12 (n=6); for samples taken at 21 day intervals r= 0.25 (n=4). There were only 2 pairs of samples taken 28 days apart (r=0.25).

The repeatability of PCV was much higher than for FEC. For samples taken 3 days apart the repeatability was 0.70 and fell to 0.59 between samples taken 28 days apart (Fig. 3). Regression analysis demonstrated that there was a significant decline in the repeatability of PCV as the interval between samples increased ($p=0.031$). For FEC, the slope was not significantly different from zero ($p=0.653$).

The repeatability of IgA and eosinophilia was also dependent on the interval between samples (Fig. 4). For IgA activity, the repeatability between samples taken three days apart was 0.76 (n=8) and fell to 0.56 between samples taken 28 days apart (n=8). The repeatability of peripheral eosinophil counts was 0.52 for samples taken 3 days apart (n=8) but fell to only 0.04 in samples

taken 28 days apart (Fig. 4). The decline in repeatability as the interval between samples increased was significant for both IgA activity ($p < 0.001$) and eosinophilia ($p < 0.001$).

The relationships among FEC, PCV, IgA activity and eosinophilia were examined in repeated measures models. In the first set of models, the response variable was FEC and the explanatory variables were the interaction between group (infected or not) and day of sampling as well as the fixed effect of PCV. The effect of the group by day interaction was significant but there was no significant association between FEC and PCV confirming the result from the simpler model that averaged over time. There was also no association between FEC and IgA activity ($p=0.954$) or FEC and eosinophilia ($p=0.232$) when the group by day interaction was fitted simultaneously ($p<0.0001$). Dropping IgA activity from the model did not produce a significant association of FEC with eosinophilia ($p=0.311$). Similarly, dropping eosinophilia did not produce a significant association between FEC and IgA activity ($p=0.661$). Finally, we examined the association between IgA activity and eosinophilia in two models. Both models included the group by day interaction. When IgA activity was the response variable, neither the group by day interaction ($p=0.095$) or the influence of eosinophilia ($p=0.172$) were significant. In contrast, when eosinophilia was the response variable, both the group by day interaction ($p<0.0001$) and the association with IgA activity ($p<0.0001$) were significant. Even when the analysis was restricted to infected kids, the group by day interaction ($p<0.0001$) and the association with IgA activity ($p=0.002$) were significant.

Table 1 shows the worm counts at necropsy. The number of adult *H. contortus* recovered from the abomasum of infected animals 52 days after infection ranged from 0 to 630 with a mean recovery of 132 ± 103 females and 118 ± 89 males (10.6% of the infecting dose). In the small intestines of the same animals, the number of *T. colubriformis* recovered ranged from 0 to 150 with a mean recovery of 27 ± 20 females and 21 ± 18 males (8.8% of the infecting dose). Both distributions of worm numbers among animals were overdispersed and right skewed; most animals had relatively low numbers of worms. We did not recover any *Oesophagostomum* species in the large intestine.

The length of male and female adult worms were measured separately. Among the 25 infected kids, 18 kids had both male and female *H. contortus.* We recovered only male *H. contortus* from 1 kid and only female *H. contortus* from 1 kid; . Five kids had no detectable *H. contortus* at

necropsy. The mean worm lengths of male *H. contortus* in the 19 animals ranged from 7 to 14.6 mm with a mean of 12.8 ± 1.8 mm while the length of female *H. contortus* ranged from 7 to 21.6 mm with a mean of 17.2 ± 3.6 mm. Among the infected kids, 9 had both male and female *T. colubriformis*; 2 kids had only male *T. colubriformis* at necropsy while 7 kids had only female *T. colubriformis*. The remaining 7 kids contained no *T. colubriformis*. The mean length of male *T. colubriformis* ranged from 4 to 7 mm with a mean of 6.1 ± 0.7 mm while the mean length of female *T. colubriformis* ranged from 6 to 7.8 mm with a mean length of 6.6 ± 0.9 mm. In both species, on average male worms were shorter than female worms.

There were no significant associations of IgA activity or peripheral eosinophilia with worm number or mean worm length for *H. contortus* or *T. colubriformis*. Similarly, neither worm was associated with the mean PCV or the FEC. We created a composite worm burden by multiplying the number of male worms by mean male length in that animal and pooling this with the number of female worms multiplied by the mean female length. Again, there was no association of the composite worm burdens for each nematode species with IgA activity, eosinophilia and PCV. The composite *T. colubriformis* burden (p=0.001) but not the composite *H. contortus* (p=0.076) was associated with the mean FEC.

Discussion

This study examined the responses of goats to a low-dose, experimental infection with a mixture of nematodes, predominantly *H. contortus* with smaller numbers of *Trichostrongylus* spp. and *Oesophagostomum* spp. The L3 recovery based on genera was 82:12:6 similar to the 75:12.5:12.5 (from the 6:1:1 ratio of the infective dose of L3) mixture used to establish the infection. Mixed infections are seldom used for experimental infections of small ruminants but they do have the advantage of providing a more realistic model of natural infection. In particular, the use of several species of nematodes makes it less likely that any unusual features of the goat response are specific to a single species of nematode.

The mixed, low dose, experimental infection may more closely imitate natural infection in situations where animals are kept indoors and reared intensively. The 6:1:1 ratio of infective larvae resembles natural infection of nematodes that commonly occurs among goats in Malaysia where typically *H. contortus* predominates [40]. In Malaysia, the prevalence of *Oesophagostomum* spp. was only 4% in comparison to *H. contortus* (56%), *Trichostrongylus* spp. (30%), *Bunostomum* spp. (7%) and *Cooperia* spp. (3%) based on faecal culture from 16 private small ruminant farms [41]. In another study, the prevalence of *Oesophagostomum* spp. was only 1% whereas *H. contortus* and *Trichostrongylus* spp. had prevalences of 73% and 26%, respectively in eight smallholder goat farms, [42].

In any biological study, there may be a discrepancy between the true and the measured response that could prevent the detection of any association. This discrepancy is particularly important when studying a potential failure to respond in a susceptible species. We used Cronbach's alpha coefficient to assess the reliability of our assays. This process indicated that the alpha coefficents for the FEC, PCV, IgA activity and peripheral eosinophilia assays had values above 0.7 and can therefore be considered as reliable [37]. This procedure does not appear to have been applied to goat parasitological assays before. However, it does offer some assurance that the observed differences between sheep and goats in response to nematode infection are not simply a consequence of poor quality assays in goats.

As expected, the infection generated significant FEC and IgA responses [43]. After the prepatent period, mean FEC rose and then fluctuated between 300 and 570 epg. In sheep aged 9-18 months the relationship between the number of adult *H. contortus* and the FEC is linear and approximately equal with a regression coefficient 0.84 + 0.66 [44]. In other words, one adult worm produces approximately one epg of faeces. In our study, one worm produced roughly two epg of faeces so there is no indication that the worms were substantially more or less fecund than expected, especially as older sheep would be expected to produce more faeces than the kids in our study (approximately 3 to 7 months old at the time of infection).

At necropsy, the mean number of *H. contortus* was 190 compared to a mean number of 26 for *T. colubriformis*. Although there were more *H. contortus* than *T. colubriformis* recovered at necropsy, FEC were only associated with the number of *T. colubriformis*. This was unexpected as *Haemonchus* spp. are more prolific egg layers than *Trichostrongylus* spp. with production of up to 5000 eggs per day per nematode [45]. As most worms were *H. contortus*, and this is the more fecund species, most eggs were probably from *H. contortus*.

There was considerable variation among animals in mean IgA activity against *H. contortus* third stage larvae but this variation was not associated with the number of adult worms recovered at necropsy or with mean worm length. Possibly genetic differences among goats have a stronger influence on the intensity of the mean IgA response than variation in the number of established worms. In sheep, IgA responses against nematodes are under strong genetic control [20].

Although there was a drop in the PCV followed by a recovery throughout 52 days of infection, there was no significant difference in PCV between the infected and uninfected animals. This indicates that the infection did not cause substantial anaemia in the infected goats. Possibly a heavier infection would have generated more severe pathology.

Perhaps the most surprising result of this study was the failure to generate a detectable eosinophil response. This is in contrast to sheep where nematode infection generates coordinated IgA and eosinophil responses [21]. Previous studies in goats have suggested that only resistant but not susceptible goats can mount eosinophil responses to infection [46]. Possibly a larger challenge would

have produced more pronounced eosinophil and IgA responses but larger doses are more pathogenic and can compromise immune responses [12].

There were substantial and significant differences among animals in the intensity of the response to infection. This supports previous studies in goats infected with *H. contortus* [6,7]. Studies in sheep suggest that much of the individual variation in susceptibility to infection is due to genetic variation [47-49]. Goats do show genetic variation in susceptibility to natural nematode infection [10] but additional studies would be needed to confirm that this was a major source of individual variation in goats.

The repeatability of the response to infection was determined for FEC, PCV, IgA activity and eosinophilia. The repeatability indicates the similarity between different measurements and provides an upper limit to the extent of genetic variation [39]. The repeatability decreased as the time between measurements increased which is similar to sheep infected with *T. circumcincta* [38]. The pattern of the decline can be used to inform the choice of variance structures for repeated measures analyses [35]. However, the values and the patterns for repeatability in goats were dissimilar to the patterns for the same variables in sheep. For example the mean repeatability of FEC taken one week apart was 0.48 in Scottish Blackface sheep [38] but only 0.28 in the Boer goats infected in our study. The eosinophil counts in sheep taken during the peak response were negatively correlated with counts taken later in the response. For example, counts taken 14 days after infection were negatively correlated (r=0.57) with counts taken 55 days after infection [38]. In this study, there was no peak in the eosinophil response to infection and the correlations were weak but positive among counts taken over the course of infection (Fig. 4).

Repeated measures analyses failed to find associations between FEC and either IgA activity or eosinophilia. This was in contrast to studies in sheep where the importance of IgA [20,25] and eosinophils [22,25] in controlling nematode infections is well established. There was an association between eosinophilia and IgA activity when eosinophilia was the response variable but not when IgA activity was the response variable. Perhaps, eosinophilia meets the requirements of the statistical model better than IgA activity; in generalised linear models the response variables can follow a variety of nominated distributions but the explanatory variables typically are assumed to follow a normal distribution. The possible association between the two variables in goats could be

a consequence of variation among individuals in the number and size of nematodes in the gastrointestinal tract following infection. Those individuals with more and larger nematodes would be expected to generate stronger immune responses. However, all infected animals were given the same dose of parasites and there was no association of worm number or worm size at necropsy with IgA activity or eosinophilia. Alternatively, as in sheep [50] there could be genetic variation in immune responsiveness to nematode infection. If some animals had genes that predisposed to strong immune responses, this could produce a positive association between eosinophilia and IgA activity.

In conclusion, a detailed analysis of the IgA and eosinophil responses to deliberate nematode infection in Boer goats in Malaysia showed that there was an increase in nematode-specific IgA activity but no detectable eosinophil response. In addition, there was no association between increased IgA activity or eosinophilia and FEC. The absence of a detectable eosinophil response and the absence of an association between eosinophil numbers or IgA activity with egg counts or worm burdens suggest that in goats, IgA and eosinophils do not act to control nematode infection. The lack of functional eosinophil receptors for IgA in goats [24] may reduce the effectiveness of the immune response against gastrointestinal nematode infection.

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List of figures:

Fig. 1. Faecal egg count (FEC) and packed cell volume (PCV) responses to experimental, mixed nematode infection. There were 25 infected kids and 4 controls. The responses of the uninfected controls are indicated as PCVctl.

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Basripuzi, N. Hayyan = conceived the study, planned and conduct the research, drafting and revised the paper, approval of the submitted and final version.

Reuben, S.K., Sharma = co-supervised the project, provided advice on protocols and experimental procedures, revised paper, approval of the submitted and final version.

Nurulaini, R = provided advice on protocols and experimental procedures, revised paper, approval of the submitted and final version.

Mehru,N = involve in field works and laboratory works, revised paper, approval of the submitted and final version.

Khalida, H = involve in field works and laboratory works, revised paper, approval of the submitted and final version.

Busin, V = provided advice on protocols and experimental procedures, revised paper, approval of the submitted and final version.

Caitlin, J. Jenvey = conceived and carried out the analyses, revised paper, approval of the submitted and final version.

Callum Cairns = conceived and carried out the analyses, revised paper, approval of the submitted and final version.

Stear, M.J. = supervised the project, planned the research, provided training, advice on protocols and experimental procedures, drafting and revised the paper, approval of the submitted and final version.

Data availability statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Table 1. Faecal egg counts and total worm counts in experimentally infected Boer goats.

Goat ID	FEC (epg) Day 52	Gastrointestinal nematodes			
		Haemonchus contortus (Number)		*Trichostrongylus colubriformis* (Number)	
		Abomasum		Small intestine	
		F	M	F	M
RB68	1325	170	210	20	10
RB41	0	0	10	0	10
RB40	425	390	240	30	0
RB47	250	90	100	0	0
RB44	0	0	0	10	0
RB67	200	190	120	20	0
RB58	450	10	0	20	10
BR57	0	0	0	0	0
RB36	125	80	40	0	0
CY22	0	10	10	20	0
CY32	0	30	30	0	0
FA31	0	150	110	0	0
FA36	100	60	160	10	0
FA32	25	80	70	10	0
FA37	200	220	180	0	10
BR08	875	50	20	30	40
BR25	50	0	0	90	60
BR17	0	290	310	20	10
BR01	150	70	120	30	10
RB64	825	160	120	50	40
CY40	50	0	0	0	0
CY26	600	0	0	0	0
CY27	150	270	260	10	10
CY30	525	110	90	30	20
BR26	50	80	40	30	0

F = female; M = male. There was no *Oesophagostomum* spp. detected in the large intestine of the goats at necropsy.

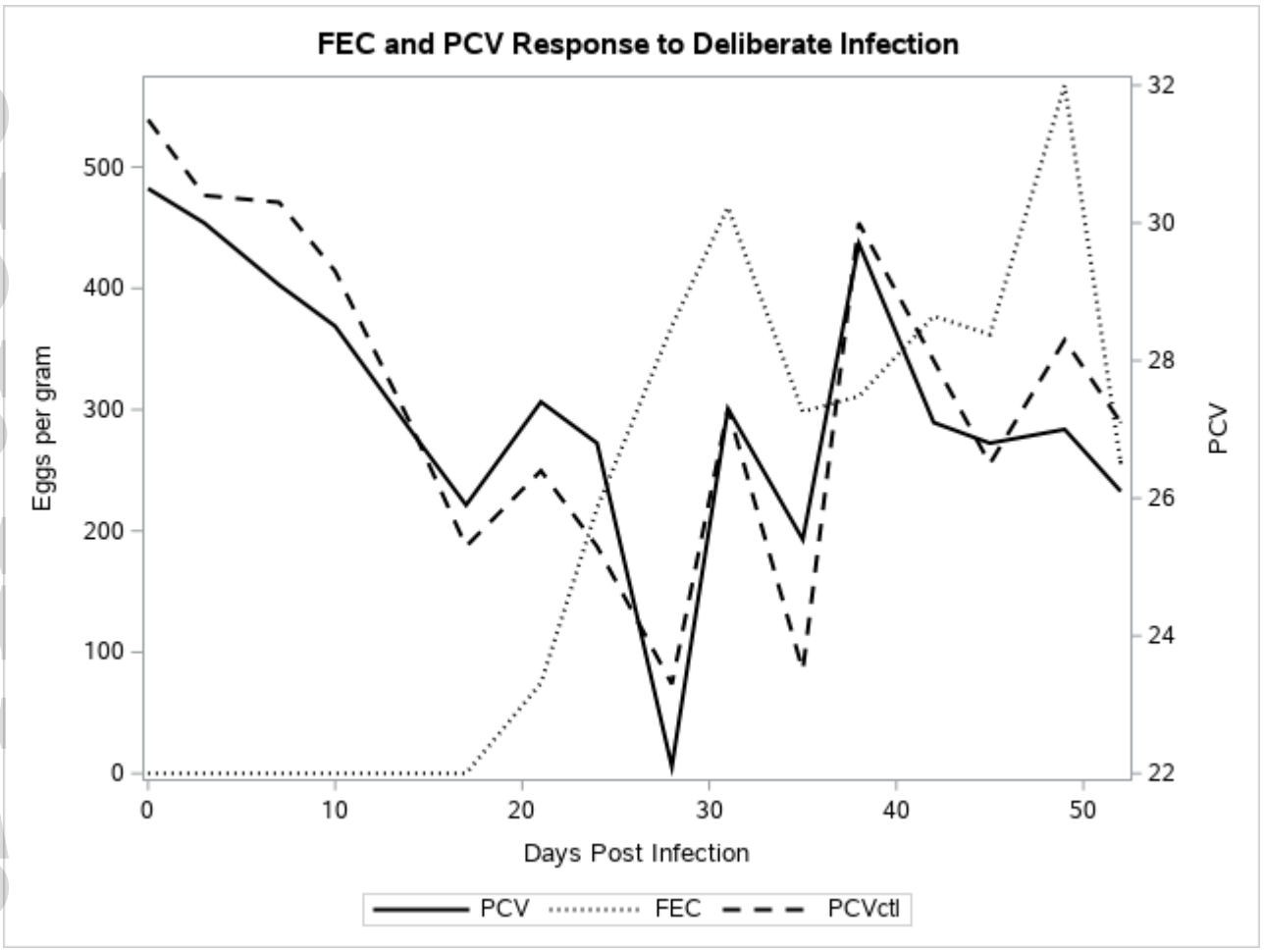

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Fig. 1. Faecal egg count (FEC) and packed cell volume (PCV) responses to experimental, mixed nematode infection. There were 25 infected kids and 4 controls. The responses of the uninfected controls are indicated as PCVctl.

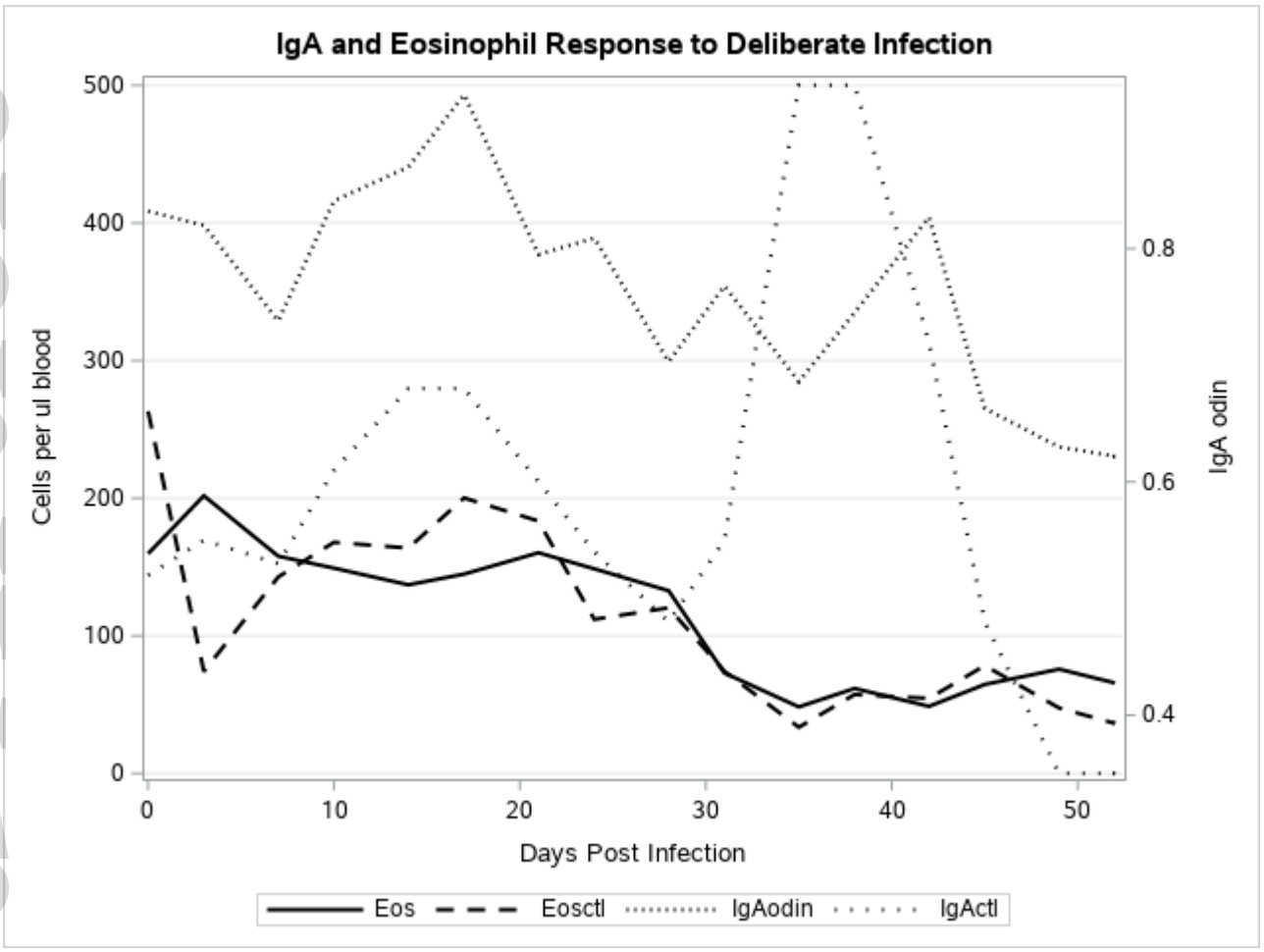

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Fig. 2. Parasite-specific immunoglobulin A activity (IgA) and eosinophil (Eos) responses to experimental, mixed nematode infection. The responses of the uninfected control animals are indicated as IgActl and Eosctl

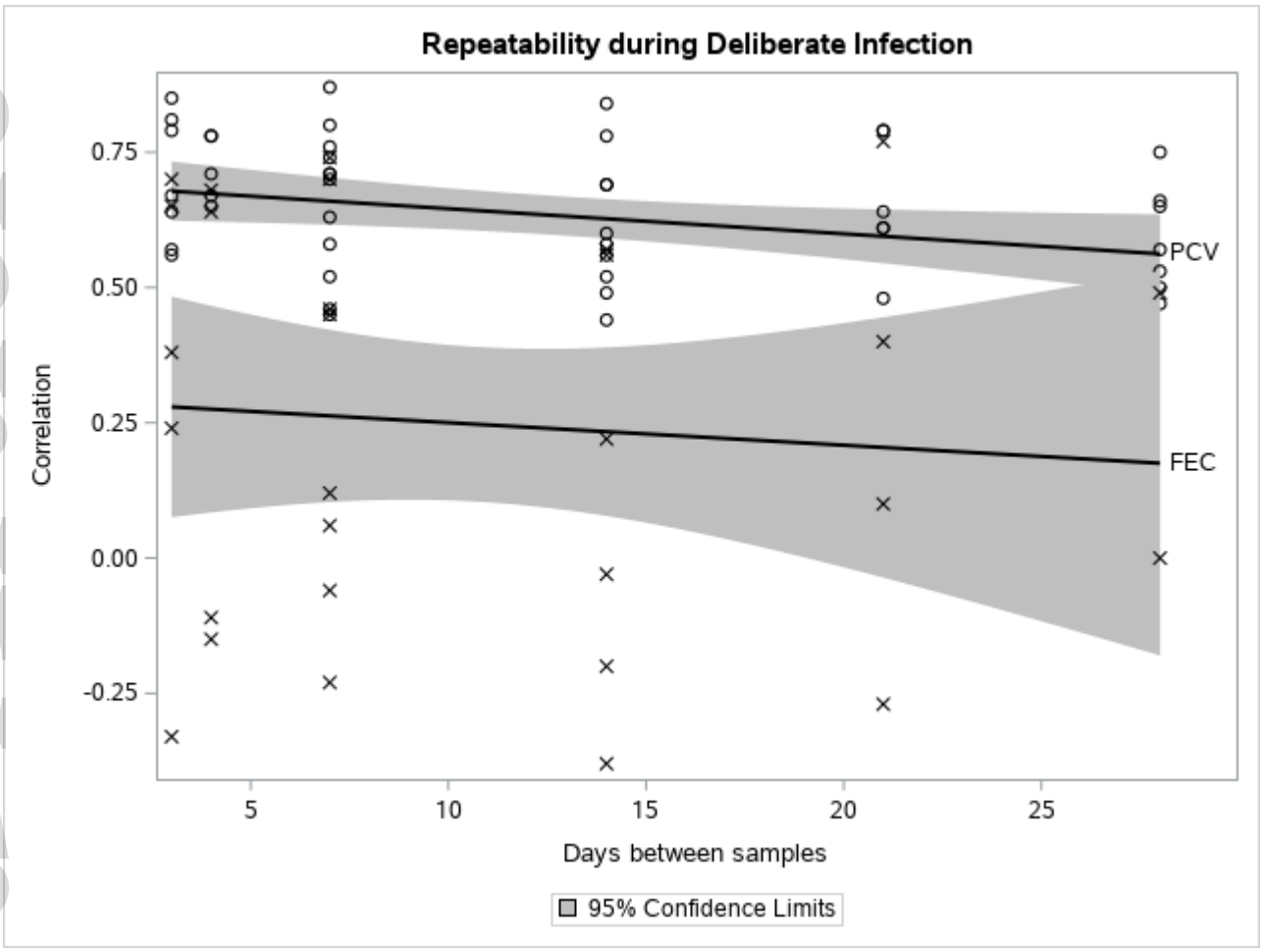

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Fig. 3. The repeatability of faecal egg count (FEC) and packed cell volume (PCV) following experimental, mixed nematode infection. The repeatabilities for FEC are indicated as crosses while the repeatabilities for PCV are shown as circles.

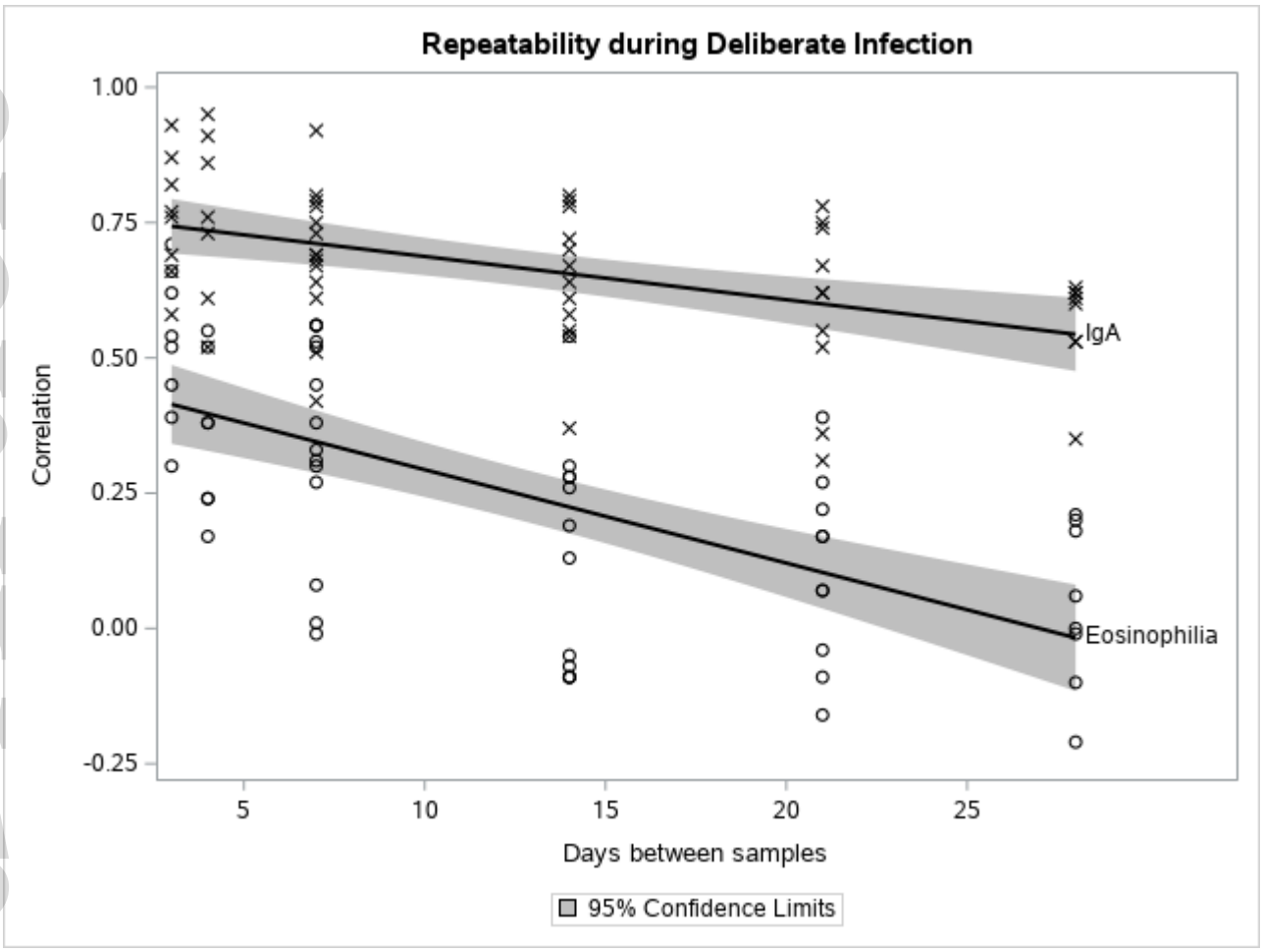

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Fig. 4. The repeatability of parasite-specific immunoglobulin A activity (IgA) and peripheral eosinophilia (Eos) following experimental, mixed nematode infection. The repeatabilities for IgA are indicated as crosses while the repeatabilities for Eos are shown as circles.