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The response of Nigerian West African Dwarf goats to experimental infections with *Haemonchus contortus*

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SUMMARY

One option for controlling haemonchosis in warm pastoral regions is improvement of resistance by selective breeding. Variation in acquired immunity to *H. contortus* and immunological correlates of infection were studied in West African Dwarf (WAD) goats. Following exposure to 5000 L3, 63 per cent of the inoculum established but 77 per cent of established worms were expelled by week 5. All infected animals were anaemic (day 14). When exposed to 2000L3, 36 per cent of the inoculum was still present (day 35) with no loss by day 49. Persisting primary infection worms survived a superimposed challenge (day 35), but their growth was slowed and resistance to challenge was significant. Most goats showed eosinophilia and parasite-specific IgG responses to primary infection, but only eosinophilia increased after challenge. No consistent associations were found between parasite burden and any immunological measures of infection, but parasite egg counts showed considerable variation. Overall, our results suggest that resistant genotypes exist among the WAD goat population.

THE West African Dwarf (WAD) goat is the indigenous breed in the humid and sub-humid zones of West Africa. It is of major economic importance to rural communities, where the vast majority of the goat population in these zones is to be found. In Eastern Nigeria, WAD goat production is essentially a subsidiary, rural agricultural activity. As a result, this vital livestock resource has remained largely under-exploited, and continues to rely heavily on entrenched inefficient traditional methods of husbandry to meet the current high demands for goat meat.

One of the most prevalent diseases, and a major cause of poor productivity in WAD goats, is parasitic gastro-enteritis (PGE) caused by several species of gastrointestinal (GI) nematodes, the most important of which are *Haemonchus contortus* and *Trichostrongylus colubriformis* (Chiejina, 1987). Although information is currently available on the epidemiology of PGE and on the broad principles of its control in this breed of small ruminants in the Nigerian humid and subhumid zones (Chiejina, 1993), there is little published research data on the immunological and genetic aspects of host-parasite relationships in *H. contortus* and *T. colubriformis* infections in these hosts.

Immuno-genetical aspects of GI nematode infections in ruminants are important and growing areas of research into alternative strategies for the control of caprine and ovine

PGE. For example, work in these two areas has resulted not only in the production of a number of candidate vaccines, such as the *H. contortus* gut membrane-derived glycoprotein antigen, H11 (Andrews et al 1995; Newton 1995; Munn et al 1997), but also in the identification of immunogenetic and parasitological parameters for the selection of parasite resistant breeds and blood lines for possible breed improvement schemes (Douch et al 1995; Bisset et al 1996; Eady et al 1996). Recent reports have also identified some parasite-resistant tropical breeds of sheep and goats and their crosses which may have a role to play in the control of PGE in small ruminants in the tropics (Mugambi et al 1996; Mugambi et al 1997; Romjali et al 1996; Mandonnet et al 1997). Of particular interest is the observation that resistant phenotypes can be identified in an out-bred and unselected flock under field conditions, using simple parameters such as faecal egg counts, circulating eosinophils and serum parasite-specific IgG and IgG1 levels (Hohenhaus and Outteridge 1995; Douch et al 1995). It is not clear whether this also applies to WAD sheep and goats, as similar studies have not been done in these animals.

We report here a pilot study of experimental infections with a laboratory strain of *H. contortus* in the Nigerian WAD goat. Our objectives were 1) to determine whether this breed, which is noted for its trypanotolerance, would develop good acquired immunity to primary and challenge infections and 2) to define the clinical, parasitological and immunological parameters associated with host resistance and resilience to infection.

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TABLE 1: Experimental design for Experiments 1 and 2

Experiment	Group	No. of L3 administered on day		Day of autopsy No. of goats killed				Remarks
		0	35	14	28	35	49	
1	A	5000	—	5	—	—	—	Primary infection
	B	5000	—	—	5	—	—	Primary infection
	C	5000	—	—	—	5	—	Primary infection
	D	—	—	—	—	6	—	Uninfected controls
2	A	2000	—	—	—	6	—	Primary infection
	B	2000	—	—	—	—	6	Primary infection
	C	2000	2000	—	—	—	7	Primary + challenge infections
	D	—	2000	—	—	—	6	Challenge controls
	E	—	—	2	—	—	6	Uninfected controls

MATERIALS AND METHODS

Experimental animals and their management

The newly-weaned (5 to 6 months old) WAD goat, straight from its village environment, is very difficult to rear successfully under total indoor laboratory conditions; however, this problem was successfully overcome in our study. The use of dry season-reared animals of this age, which usually harbour negligible or no GI nematode infections (Chiejina 1986; Chiejina et al 1989) and the procedures described below, in preparing and housing the animals, ensured that no extraneous GI nematode infections took place.

Twenty-five and forty male WAD goats aged approximately six months were purchased at the end of the dry seasons (March/April) of 1996 (Experiment 1) and 1997 (Experiment 2) respectively, from selected village goat keepers. Each animal was weighed and a faecal sample collected for coccidia oocyst and worm egg counts and culture for the identification of strongyle infective larvae (L3). Blood samples were also collected and screened for the presence of trypanosomes. The animals were individually dusted with the insecticide coumaphos (Asuntol powder, Bayer, Leverkusen) against ectoparasites, treated with the anthelmintic fenbendazole (Panacur, Hoechst) at 7.5 mg kg⁻¹ body weight and a coccidiostat containing 200 mg Trime-thoprin B.P. and 100 mg Sulphadiazine B.P. (Chanoprin bolus, Chanelle Pharmaceutical Manufacturing Co. Ltd., Ireland), as recommended by the manufacturers.

Those animals which were parasitologically negative on faecal and blood examinations (21 in Experiment 1 and 37 in Experiment 2) were quarantined for three weeks, during which daily observations of their clinical condition and weekly records of faecal worm egg counts, packed cell volume (PCV), body weight, circulating eosinophil and total red blood cell (RBC) counts were obtained. This period was also used to allow the animals to adapt to the managerial and feeding routines in the animal house.

At the end of the quarantine period all animals received a second dose of anthelmintic and 24 hours later were moved to the experimental animal house, which was provided with concrete floors and walls. A week later (Day 7), they were assigned to their respective pens which had been washed, dried and bedded with dry straw one month earlier. Fresh straw was added weekly to ensure dry conditions in the pens. The animals were fed ad libitum, in troughs, with a concentrate mix consisting of two parts of ground palm kernel cake and one part each of ground rice bran and broiler grower's mash (19 per cent crude protein) and twice daily

with cut-and-carry grass and shrubs harvested from experimental plots known to be inaccessible to livestock. Water was also available ad libitum.

Haemonchus contortus

A sheep strain of this nematode was obtained from the Moredun Research Institute, Edinburgh, Scotland. This strain was originally isolated from sheep in Kenya in the 1950s and it has been passaged about twice/annum since then in sheep maintained at the Moredun Institute. It was subsequently passaged once in WAD goats from which faecal cultures were prepared and L3 harvested according to MAFF (1977).

Experimental infections

The L3 were used within 21 days of recovery from faecal cultures. They were administered by stomach tube on Day 0 (D0) and according to the protocol shown in Table 1.

Faecal worm egg count

In Experiment 1, faecal worm egg counts were carried out weekly, between D0 and D14 and daily thereafter, on pooled freshly passed faeces collected in the morning of the day of examination from the appropriate group, using the salt floatation technique. In addition, samples were taken from individual animals at autopsy. Those samples which yielded counts ≥ 100 eggs per gram of faeces (EPG) were further examined using a modification of the McMaster method (MAFF, 1977). In Experiment 2, individual faecal samples were examined weekly between D0 and D21 and twice a week, thereafter.

Worm count

At autopsy the abomasum was isolated and its content, mucosal washing and scraping were collected and processed for worm counting, according to MAFF (1977). Ten per cent aliquots of the abomasal content and washing and the whole of the mucosal digest were examined and the worms present counted, sexed and aged. In the second experiment, all worms were carefully examined and assigned to larval or adult stages on the basis of morphological development and size, and 15–20 adult male and female worms from each of the animals in groups A, B and C were also measured, as described by Behnke et al (1997).

Body weight measurements

Body weights were determined weekly.

Haematology

PCV was measured and total RBC counts were carried out weekly on fresh blood, collected with EDTA as anti-coagulant, to monitor the degree of anaemia arising from infection. RBC count and PCV were done according to Dacie and Lewis (1995). As a quality control we examined the relationship between RBC counts and PCV of groups A, B, C, D and E on days 35 and 49 in Experiment 2. As expected, on both days there was a highly significant positive relationship (D 35, $R = 0.796$, $n = 29$, $t = 6.83$, $P = 0.001$; day 49, $R = 0.787$, $n = 23$, $t = 5.85$, $P < 0.001$). Circulating eosinophil counts were carried out by Carpentier's method (Dawkins, Windon and Eagleson 1989).

Serum IgG

One millilitre blood samples were collected from each animal weekly. Serum was separated and stored at -20°C . *H. contortus*-specific serum IgG levels were measured using a standard ELISA technique (Schallig et al 1994), at a previously determined optimum *H. contortus* E/S antigen concentration ($1.5 \mu\text{g ml}^{-1}$), a serum dilution of 1/1600 and IgG-Alkaline phosphatase conjugate dilution of 1/4000. Known naive and positive sera were included in every test plate. IgG levels were measured as optical density (OD) at 405 nm, using a Dynatech MR200 reader. The OD was further corrected, according to the following formula (Woodford et al 1990):-

$$\text{Corrected OD index} = \frac{(\text{PS Day 0}) - (\text{NS Day 0})}{(\text{PS Day T}) - (\text{NS Day T})} \times (\text{TS}) - (\text{NS Day T}),$$

PS = positive control serum OD.

NS = naive serum OD.

TS = test serum OD.

Day 0 = day against which test values were standardised.

Day T = day test was done.

Statistical analysis

Data are presented as group mean values \pm standard error (SEM). Parametric tests (one or two-way repeated measures ANOVA using type III sums of squares) were used where data, transformed where necessary, met the required assumptions and were carried out using SPSS (Version 7.0 for Windows 95). Tests of correlations between variables were carried out using parametric procedures on normalised data-sets. Where the data were not normally distributed and could not be normalised, non-parametric statistical procedures were used and summary statistics were presented as geometric means of transformed values [$(x + 1)$ for eosinophils and $(x + 0.0001)$ for optical density index] to enable zero value to be included. When a difference was expected in a specific direction, a priori hypotheses were tested by the integrated procedures (equivalent to the Jonckheere test for ordered alternatives, see Siegel & Castellan, 1988) described by Meddis (1984) and the test statistic z is given as appropriate;

in the event of a specific hypothesis being rejected ($P = \text{not significant [NS]}$), groups were further compared by a general non-parametric one-way ANOVA (equivalent to the Kruskal-Wallis test) in order to determine whether there were any significant differences between the groups, and the statistic H is given. In the case of non-parametric correlations, one-tailed tests were used when a priori predictions about the direction of the change with time were possible but otherwise two-tailed tests were employed and the statistic r_s is given for both. Probabilities (P) of 0.05 or less were considered significant.

RESULTS

Experiment 1

Parasitology

The dose of 5000 L3 proved too high for some animals and four severely affected animals were killed on D11, 12, 18 and 25. The data for the first two were included with those of the D14 group and the data for the remaining two were included with the D28 group in the mean values in Fig 1, although individual data points are also shown. The worm burdens of all the animals are shown in Table 2.

Figure 1 shows that a high proportion of the inoculum established, with 62.8 per cent surviving to D14, indicating that the animals were highly susceptible to infection with this isolate of *H. contortus*. The percentage of female worms varied from 51.5 to 54.5 per cent. However, the worm burdens in group B, killed 18–28 days after infection, were lower (overall reduction of mean worm burden relative to group A = 76.8 per cent) with only one animal harbouring worm burdens equivalent to those in group A. Two of the goats in group B (Nos. 110 and 117) had lost virtually all of their worms (Table 2). The worm burdens on D35 were similar to those on D28. We tested the a priori prediction that

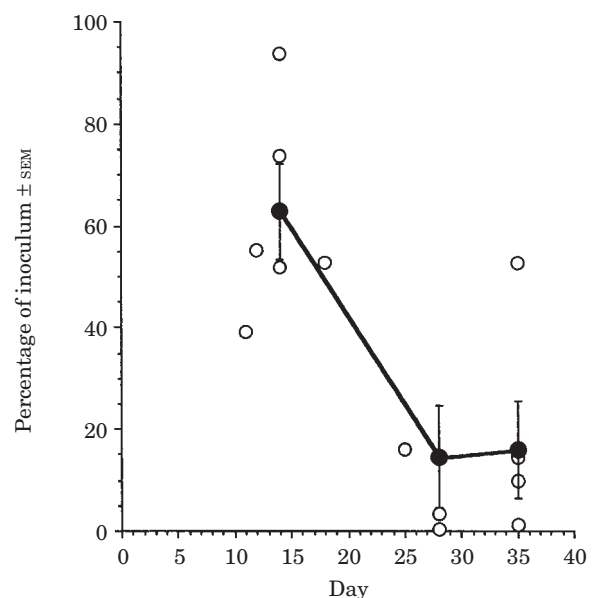


FIG 1: Experiment 1. The mean worm recovery (●) and the total worm burdens (○) of individual goats on days after infection. For statistical analysis see text.

TABLE 2: Worm burdens of all goats at autopsy in Experiment 1

Group No.	Reference	Day killed ¹	No. of worms			Per cent females
			Males	Females	Total	
A	102	11d	950	1010	1960	51.53
A	105	12d	1280	1350	2760	53.62
A	103	14	1780	1910	3690	51.76
A	109	14	1250	1350	2600	51.92
A	111	14	2133	2560	4693	54.54
Mean			1478.6	1636.0	3140.6	52.7
±SEM			211.0	272.4	476.6	0.6
Median			1280	1350	2760	51.9
B	122	18d	1620	1020	2640	38.64
B	121	25d	520	280	800	35
B	108	28	80	90	170	52.94
B	110	28	20	0	20	0
B	117	28	10	0	10	0
Mean			450.0	278.0	728.0	25.3
±SEM			307.3	192.4	499.5	10.8
Median			80	90	170	35.0
C	101	35	20	40	60	66.67
C	104	35	580	140	720	19.44
C	107	35	340	160	500 ²	32.00
C	113	35	1120	1520	2640	57.58
C	114	35	60	0	60	0
Mean			424.0	372.0	796.0	35.14
±SEM			201.4	288.6	478.4	1.22
Median			340	140	500	32.0
D	123	35	0	0	0	0
D	126	35	0	0	0	0
D	130	35	0	0	0	0
D	136	35	0	0	0	0
D	139	35	0	0	0	0
D	140	35	0	0	0	0
Mean			0	0	0	0
±SEM			0	0	0	0
Median			0	0	0	0

¹ Goats which were killed earlier than the day on which it had been intended to autopsy the group are marked with d, and the day on which they were killed is recorded.

² This animal also yielded 60 immature worms

there would be a reduction in total worm burdens (group A>B>C) over the five-week period and found that $z = 2.09$, $P = 0.018$, which confirmed our expectation.

Worm eggs were first detected in faeces on D26, but counts remained very low and on D35 only three of the surviving animals, Nos. 101, 104 and 113, had counts of 1, 1 and 250 EPG respectively.

Haematology

The mean PCVs of the two groups killed on D35 are shown in Fig 2. Since PCV values were normally distributed, the data were tested by two way ANOVA, with infection (two levels corresponding to infected and not infected animals) and time (eight levels corresponding to days 0, 7, 14, 21, 28 and 35) as fixed factors, and goat (a random factor) as a repeated measure. While there was no significant main effect of infection on PCV ($F_{1,9} = 2.85$, $P = \text{NS}$), PCV changed significantly with time (main effect of time, $F_{5,5} = 21.28$, $P = 0.002$), indicating that both infected and non-infected animals showed a significant change in PCV over the course of the experiment. The significant interaction between infection and time ($F_{5,5} = 10.35$, $P = 0.011$) confirmed that PCV declined more steeply among the infected than in the control animals.

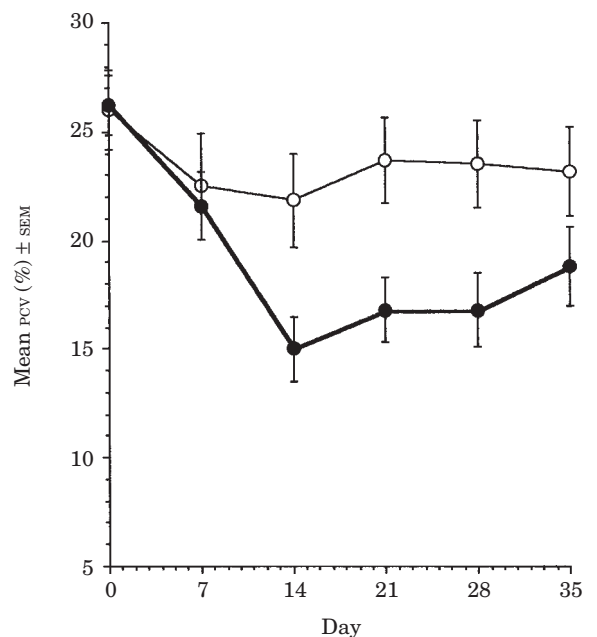


FIG 2: Experiment 1. Changes in the packed cell volume (PCV) of infected (●) and uninfected (○) goats from Groups C and D finally killed on day 35 pi. For statistical analysis see text.

TABLE 3: Experiment 2. Worm burdens in goats thirty five and forty nine days after primary and fourteen days after secondary infections

Goats		Mean worm burden \pm S E M (Range)				
Group (day of the experiment)	No.	L4	Immature L5	Total immature worms	Adult worms	Total
(A) Primary infection only (killed day 35)	6	26.7 \pm 12.6 (0–60)	43.3 \pm 13.1 (10–100)	70.0 \pm 23.4 (20–180)	658.3 \pm 172.9 (40–1350)	728.3 \pm 194.8 (60–1530)
(B) Primary infection only (killed day 49)	6	15.0 \pm 13.1 (0–80)	11.7 \pm 6.5 (0–40)	26.7 \pm 12.3 (0–80)	486.7 \pm 125.4 (140–980)	513.3 \pm 133.3 (140–1020)
(D) Challenge infection only (killed day 49)	5	12 \pm 12 (0–60)	150 \pm 61.2 (0–350)	162.0 \pm 54.6* (60–350)	0	162.0 \pm 54.6 (60–350)
(C) Primary + challenge (killed day 49)	7	11.4 \pm 4.0 (0–20)	2.9 \pm 2.9 (0–20)	14.3 \pm 3.7* (0–20)	328.6 \pm 139.9 (40–1100)	342.9 \pm 137.8 (60–1100)

Groups D and C were killed on day 49 of the experiment, corresponding to 14 days after the secondary infection administered on day 35.
 *Groups compared statistically Meddis 1-way ANOVA, $z = 2.952$, $n = 5, 7$, $P = 0.002$

Experiment 2

Parasitology

Table 3 summarises the worm recoveries from groups A, B, C and D. No worms were recovered from group E (uninfected) confirming that no background transmission of GI nematode infection had occurred during the experiment. Group A ($n=6$) was necropsied 35 days after the primary infection and had a mean worm burden of 728.33 ± 194.75 (range, 60 to 1530). This was marginally, but not significantly ($H = 0.78$, $P = NS$) higher than the mean worm burden of group B (513.3 ± 133.3) killed two weeks later. D35 worm burden represented a mean percentage survival of 36.4 ± 9.7 per cent of the inoculum, higher than in Experiment 1, and comprised 3.7 per cent L4, 5.9 per cent L5 and 90.4 per cent adult worms. The percentage of female worms (L5 + adults) was 55.6 ± 4.0 per cent on day 35, much in line with Experiment 1. There was no significant change over the following two weeks (D49 % females = 49.2 ± 5.29 ; $H = 0.52$, $P = NS$).

The faecal egg count (FEC) data are shown in Fig 3. The pre patent period varied from approximately 23–28 days. Six goats were patent on D24, 13 on D26 and 15 on D28. All the goats were passing worm eggs by D33, which was the day of peak egg output for virtually all the animals.

Until D35, all the 18 goats in the three groups illustrated (A, B and C), underwent identical treatments and yet there was wide between- and within- group variation in the egg counts. Eight of the goats had peak counts ≥ 2000 ; four 500–1000 and six < 500 EPG. Of the six lowest egg producers, three had counts of only 50 EPG or lower and two other animals in this category (No. 317, group B and No. 323, group C), which survived to D49 maintained the low egg production.

The wide variation meant that presenting the data only by mean values would obscure interesting differences between individual animals, hence the data for individuals within treatment groups have been used in Fig 3. As can be seen from these data, Group A had two individuals with EPG greater than 1000, while B and C three such individuals each. In group B, which had no further experimental treatment, egg counts dropped by D37 in all the animals, although there was a second resurgence of egg production by week 6 in two of the high producers (EPG > 1000) and one low producer.

Fig 4 gives the mean lengths, \pm SEM, of adult male and female worms from each animal and for all animals within each group, for groups A and B (primary infection) and

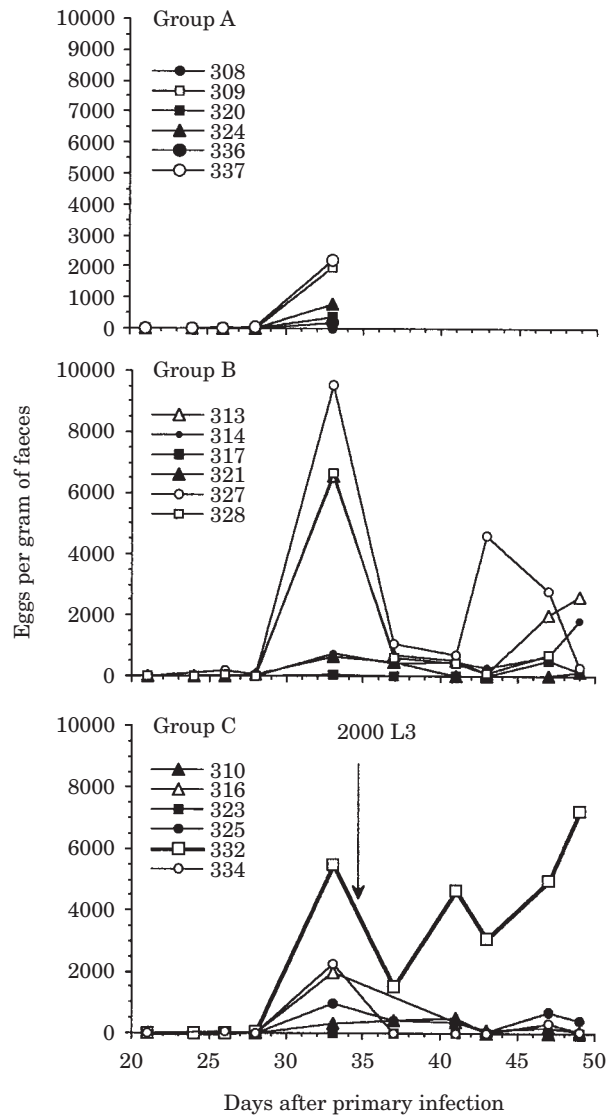


FIG 3: Experiment 2. Faecal egg counts during the course of primary and secondary infections with *Haemonchus contortus* in individual goats from Groups A (primary infection only, killed on day 35 pi), B (primary infection only, killed on day 49); and Group C (primary and challenge infections). Each symbol represents a specific animal. The solid symbols represent goats whose egg counts never exceeded 1000 egg. The open symbols represent goats which showed eggs > 1000 epg.

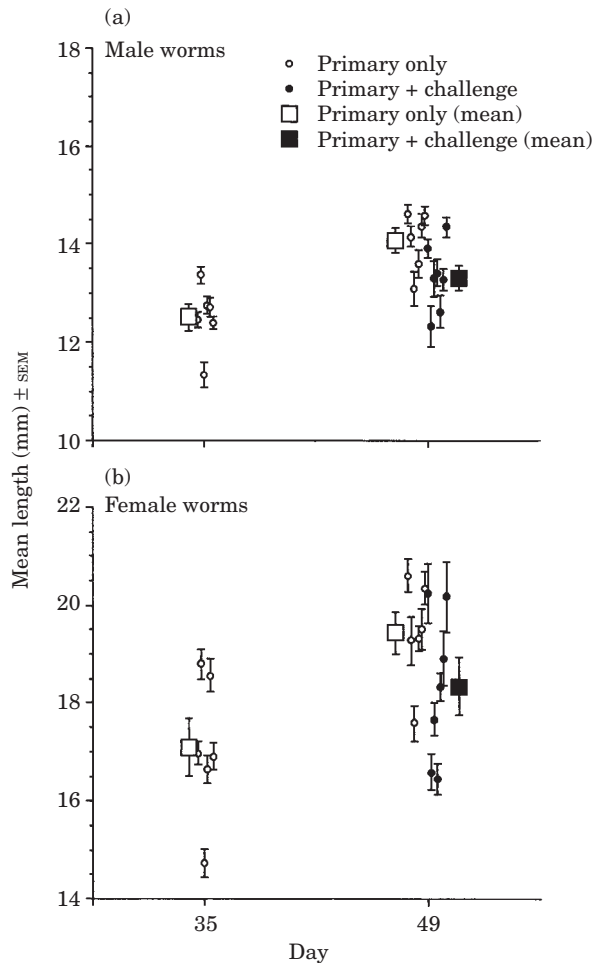


FIG 4: Experiment 2. The length of male (a) and female (b) worms recovered from animals in groups A and B (primary infection only) and C (primary and challenge infections). The small symbols represent the mean length \pm SEM. of the individual worms in specific animals. The large symbols are group mean length \pm SEM. Open symbols are groups A (day 35) and B (day 49) and solid symbols are Group C. For statistical analysis see text.

C (primary and challenge infections). On D49, there was a highly significant main effect of sex ($F_{1,22} = 154.8$, $P < 0.001$) and a weaker but nonetheless significant effect of group (Group B and C, $F_{1,22} = 4.92$, $P = 0.038$) but no significant interaction. Therefore, the worms of both sexes were significantly smaller in group C relative to group B. Further analysis showed that there was also a significant relationship across individual goats between average male and female worm lengths in group B ($r_s = 0.943$, $n = 6$, $P = 0.005$; i.e. goats with longer males also had longer females) but not in group C ($r_s = 0.5$, NS; because some goats had female worms which were shorter than expected) suggesting that the effect on growth was more severe on females than on the males in the latter group.

Acquired host-protective immunity

The four groups (B, C, D and E) killed on D49 (14 days after challenge), enabled direct assessment of acquired resistance to challenge infection to be made, since the majority of challenge infection worms could be readily distinguished by size and earlier stage of development from the

mainly adult, fecund worms persisting from the primary exposure. However, there was some delayed development of worms from the primary infection, a mean of 26.7 immature worms being recovered from group B, and these worms could not be distinguished from those developing from the challenge inoculum in groups D and C. Importantly, animals which had experienced both primary and secondary infections (group C) had even lower immature worm burdens than group B, so it is unlikely that the following interpretation was significantly compounded by persistence of immature primary infection worms in group C.

It is clear that there was a significant reduction ($z = 2.952$, $P = 0.002$) in immature worm numbers (L4 + L5) in group C animals, compared to the challenge controls (group D) even though the latter animals, which were approximately nine months old when they were challenged, showed relatively poor establishment (6.8 per cent). While the immunised and challenged animals (group C) resisted the challenge, they were unable to reject a substantial proportion of the adult worms persisting from the primary, immunising inoculum, the adult worm burden declining by only 33.2 per cent relative to group D. Nevertheless, there is some suggestion that some animals in this group had rejected the majority of their adult worms, worm burdens of only 40 (No. 334) and 60 (No. 323) being lower than that in any of the animals which had not experienced challenge (group B).

Figure 3 shows that, with one exception, faecal egg counts declined in the goats in group C after administration of the challenge inoculum. However, goat 332, originally classified as a high egg producer on the basis of D33 FEC, showed a consistent rise in egg counts through to D49. Interestingly, this animal had the heaviest adult burden (1100 worms) but showed complete resistance to challenge infection. All the others, irrespective of whether high or low egg producers, showed declining FEC towards D49 and in none did the count rise above the 1000 EPG cut-off point subsequently.

Body weight

Figure 5 shows the percentage change in body weights relative to D0. There was a significant gain in weight across treatment groups from D0 ($F_{7,12} = 13.96$, $P < 0.001$). Despite the slight setback in the primary and challenge infection group in week 1 and subsequently between weeks 5 and 7 in this and the challenge control group, there was no significant effect of treatment group nor a significant interaction between time and treatment.

Erythrocyte counts and packed cell volume

The RBC counts and PCV are shown in Fig 6. Statistical analysis was by two-way ANOVA, with treatment group (four levels corresponding to groups B,C,D and E) and time (with eight levels corresponding to days 0, 7, 14, 21, 28, 35, 42, and 49) as fixed factors and goat (a random factor) as a repeated-measure for both parameters. In general, all groups showed declining PCVs from D0 onwards and this main effect of time was highly significant across the four groups ($F_{7,11} = 8.36$, $P = 0.001$). There was also a significant main effect of treatment group ($F_{3,17} = 4.9$, $P = 0.012$), confirming that infection had induced significant anaemia, but no

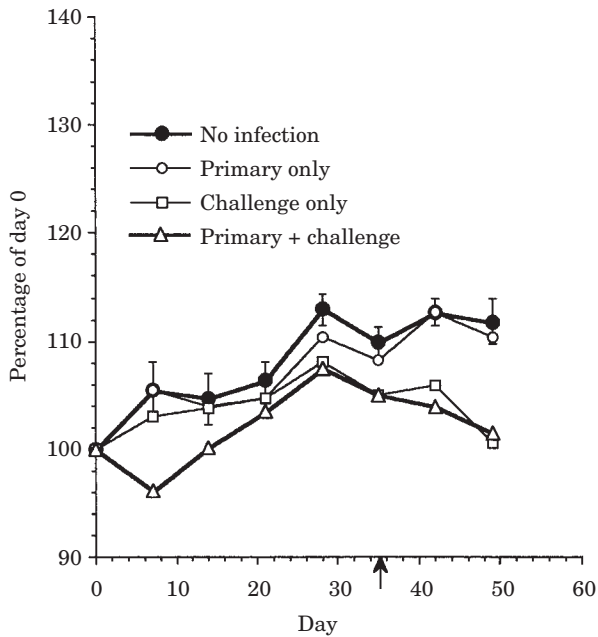


FIG 5: Experiment 2. The change in weight during the course of the experiment in groups B, C, D and E. The data are expressed as the mean of the weights of individual animals calculated as the percentage of the weight on day 0. For clarity S.E.M.s are only shown for the naive control group E. For statistical analysis see text. The arrow indicates the time when the challenge infection was administered to groups C and D.

interaction between treatment group and time. The changes in RBC counts were very similar to changes in PCV. Again there was a highly significant main effect of time ($F_{7,11} = 15.14$, $P < 0.001$). The main effect of treatment group was only just outside significance ($F_{3,17}$, $P = 0.055$) but the interaction between time and treatment group was significant ($F_{21,32} = 1.94$, $P = 0.044$).

Relationships between measures of anaemia and parasite burden

A negative relationship was expected between adult worm burden and degree of anaemia. Figure 7 illustrates this relationship, based on measurements of PCV and adult worm burdens from all four groups (B, C, D and E) killed on day 49 in Experiment 2. The regression (Fig 7) was highly significant ($t_{20} = -4.445$, $n = 22$, $P < 0.0003$). A similar analysis based on RBC gave $t_{20} = -4.521$, $n = 22$, $P < 0.0003$ (Estimate of slope = $-7.560 \times 10^{-3} \pm 1.681 \times 10^{-3}$).

Eosinophil responses

Statistical analysis of the whole data set showed that the main effect of time was not significant but that of treatment was just within the cut-off value for significance ($F_{3,16}$, $P = 0.048$) indicating that there were some differences between treatment groups. These arose because of the primary responses in some of the goats in groups B and C, the secondary responses in group C and the relatively stable eosinophil counts over the period of infection in groups D and E (Fig 8). There was no significant interaction between time and treatment ($F_{21,29} = 0.6$, $P = \text{NS}$). However, as can be seen from Fig 9, there was considerable variation

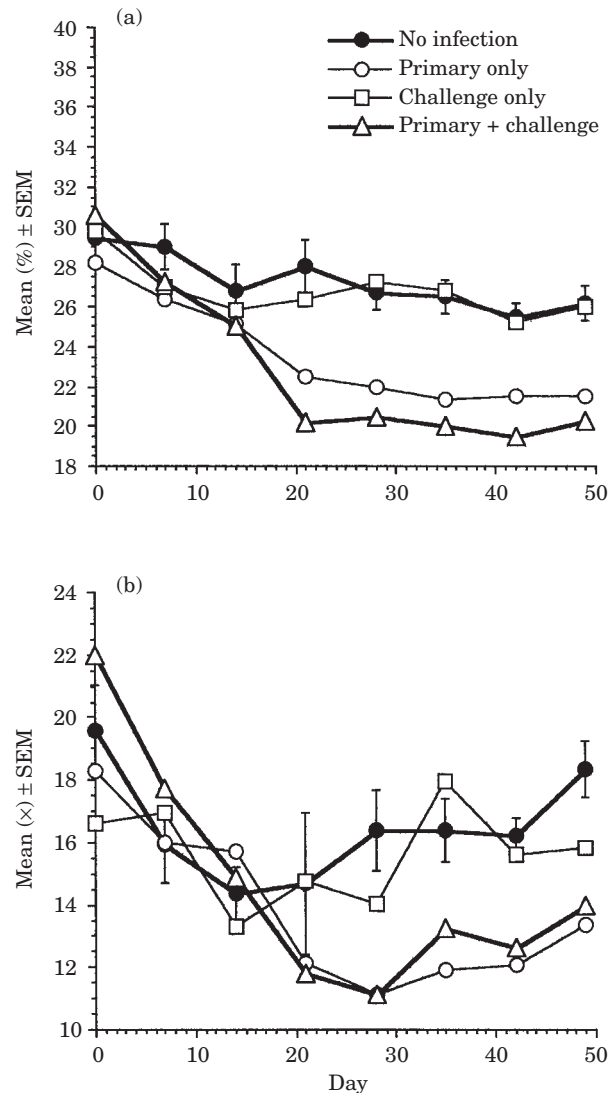


FIG 6: Experiment 2. Changes in the packed cell volume (PCV) (percentage) (a) and the red blood cell (RBC) concentration (erythrocytes $\times 10^{12}/l$) (b) of Groups B, C, D, and E. For clarity SEMs are only shown for the naive control group E. For statistical analysis see text.

within experimental treatment groups. In order to provide some indication as to when specific animals responded we calculated the upper limit for 99.7 per cent of estimates from uninfected animals. The mean eosinophil count for the eight measurements on each of 5–6 goats from the naive, uninfected control group E, on all the days on which blood samples were taken ($n = 42$) was $104.55 \text{ ml}^{-1} \pm 76.49$ (SD) and the mean $+ 3 \times \text{SD} = 334 \text{ cells ml}^{-1}$. On this basis, following primary infection only three of the six goats in group B and four of the six in group C produced a primary eosinophilia exceeding $334 \text{ cells ml}^{-1}$, although the intensity of this response in the latter group was smaller than in the two strong responders (Nos. 321 and 317) in the former. Group D, which remained uninfected until day 35, initially showed a similar pattern of eosinophilia to the uninfected controls (group E) but following infection on D35, one goat, No. 312, showed a primary response exceeding $334 \text{ cells ml}^{-1}$. In group C, which received both primary and challenge infections, five of the six goats showed good secondary eosinophilia.

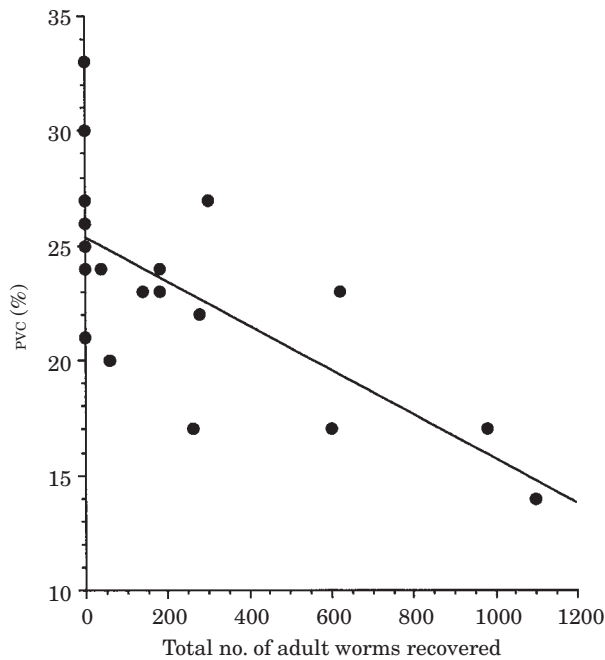


FIG 7: Experiment 2. The relationship between the PCV and the total number of adult worms recovered on day 49. The data include all animals from groups B, C, D and E. Estimate of slope = $-9.586 \times 10^{-3} \pm 2.156 \times 10^{-3}$. For further statistical analysis see text.

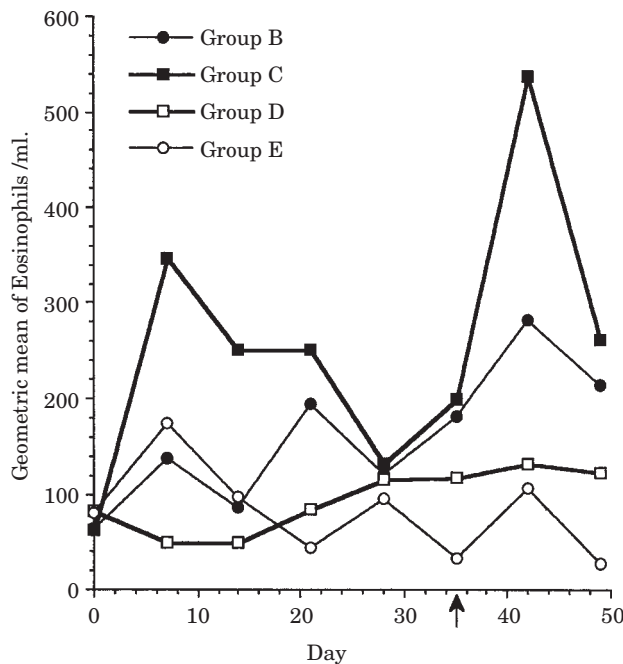


FIG 8: Experiment 2. Geometric mean values of eosinophil counts in groups B, C, D and E throughout the experiment. Error bars are not included for clarity but for details of individual variation see Fig 9. The arrow indicates the time when the challenge infection was administered to groups C and D.

IgG antibody responses

IgG levels also showed considerable variability and so, data analysis and interpretation followed much the same approach as for eosinophil counts. Fig 10 summarises the geometric means of the optical density index and Fig 11

shows the patterns of individual antibody responses in each group. The main effects of time and group were highly significant ($F_{7,11} = 8.64$, $P = 0.001$; $F_{3,17} = 7.85$, $P = 0.002$, respectively), but there was no significant interaction between time and treatment group. Thus there was a significant difference between the treatment groups with groups B and C showing overall markedly higher optical density values than groups D and E from the second week of infection onwards (Fig 10). The mean OD of uninfected goats (eight values from each of six goats, $n = 48$), was 0.019 ± 0.036 . The upper limit for 99.7 per cent of estimates based on these values (mean + 3 SD) was, therefore, 0.127. This value was used cautiously to identify times when individual animals responded.

There was clearly some background activity, as adjudged by the OD values of naive animals and group D prior to infection. However, only one animal from the control group and two animals from group D before infection, exceeded the upper limit between days seven and 14 (Fig 11). Of the 6 goats in group B, 5 produced significant antibody responses but the values for goat No. 317 never exceeded the control limit. Having achieved their peak values, the high antibody levels in four animals in the latter group persisted, with some fluctuations, until the end of the experiment. The pattern of response was quite different in group C, which, until D35, had received identical treatment to group B. Here, one animal, No. 310, showed a sustained rise in antibody level from D7 to the end of the experiment, three animals barely exceeded the control limit at various time points and one of these, No. 332, showed a strong but transient rise on the day of challenge infection. There was no evidence of a secondary antibody response in any animal following challenge infection.

DISCUSSION

Our data suggest that the animals used in the two experiments were very susceptible to the laboratory strain of *H. contortus* and developed significant anaemia, especially at a dose of 5000 L3. There is evidence that Nigerian WAD goats are highly susceptible to naturally acquired infections (Chiejina, 1986, 1987) but there are no published data on the responses of these animals to experimental infection. Therefore, it is not clear whether the severe pathology associated with this relatively moderate dose in some animals was a reflection of the well known susceptibility of goats to GI nematodes (Le Jambre and Royal 1976; Le Jambre 1984; Pomroy, Lambert and Betteridge 1986) and hence, typical of this breed, or the result of using an 'exotic' strain of the parasite to which they had never previously been exposed. It is interesting to note that workers in India (Yadav, Grewal and Banerjee 1993) and Kenya (Waruiru et al 1994) have used doses of 10,000 L3 in experimental primary and challenge infections in 4 to 5-month-old lambs and kids, respectively. Rahman and Collins (1990) infected twelve 2 to 3 month-old kids, mainly Saanens and Nubian breeds, with 40,000 L3 each, apparently without major pathological effects. Experiments using a local strain of the nematode should help to shed light on this question.

Despite their undoubted susceptibility to infection, there was evidence in Experiment 1 that many of the goats were

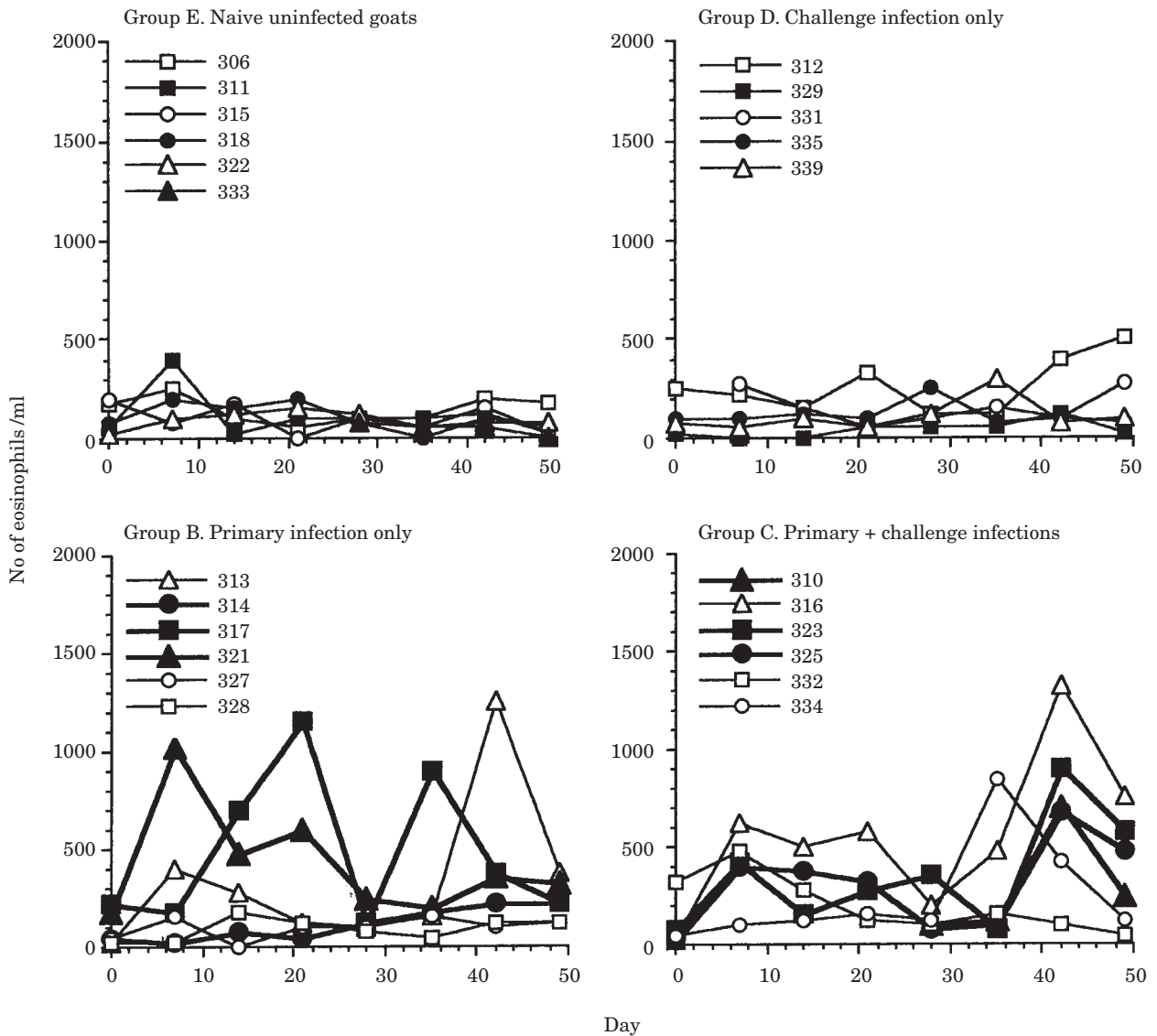


FIG 9: Experiment 2. Changes in the eosinophil concentration in peripheral blood in individual animals from groups B, C, D and E throughout the experiment. The solid symbols are animals identified earlier, on the basis of egg counts, as relatively resistant to infection and the open symbols are animals identified as susceptible to infection. See text and Fig 3 for full explanation and statistical analysis.

able to expel a large proportion of their D14 worm burden as early as D28 after the primary infection, contrasting with the chronic infections usually associated with this parasite in both goats and sheep. The overall reduction in mean worm burden over this period alone was 76.8 per cent and the reduction in total worm burden over the whole period of the experiment was significant. This was in marked contrast to the kinetics of worm loss, following primary infection in Experiment 2, where a lower inoculum had been employed. Not only did a good proportion (25.67 per cent) of the inoculum survive to D49, but three of the six animals had worm burdens ranging from 620–1020. The other three, like their counterparts in Experiment 1, had expelled a substantial proportion of their primary burden. It was also clear in Experiment 2 that challenge infection on D35 in group C did not significantly influence survival of the primary immunising infection. There were two exceptions, goats Nos 316 and

334, in which low worm burdens on D49 were preceded by a very sharp drop in faecal egg counts between D35 and D49. This response which resembled self-cure (Stoll 1928; Stewart 1955), was probably not brought about by the challenge infection, since the faecal egg counts of goat No. 328 from group B, which was not challenged, showed an even steeper fall over the same period.

Two other manifestations of acquired immunity were demonstrated in group C of Experiment 2 namely, resistance to challenge infection and stunting of growth of both adult male and female worms recovered on D49. The effect on growth was more pronounced in female worms than in males. The ability of WAD goats to resist challenge infection with this nematode and the possible existence of marked individual variations in this attribute are interesting observations which need further investigation. Furthermore, it appears that separate host-protective mechanisms are

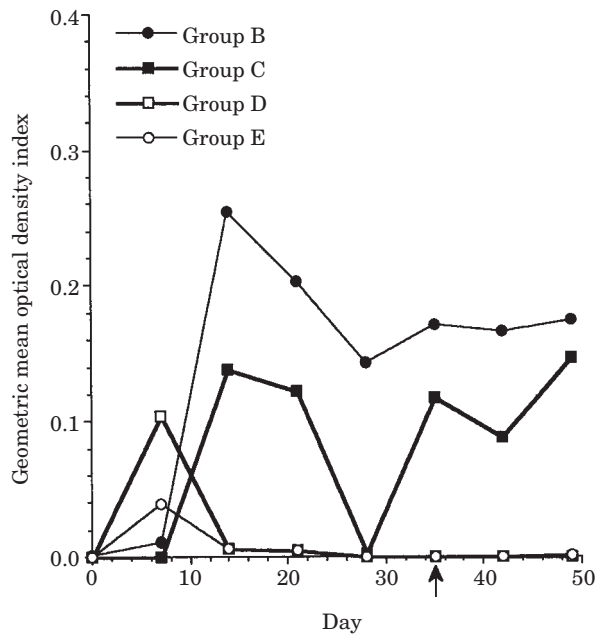


FIG 10: Experiment 2. Geometric mean values of anti ES IgG antibody concentrations in groups B, C, D and E throughout the experiment. Error bars are not included for clarity but for details of individual variation see Fig 11. The arrow indicates the time when the challenge infection was administered to groups C and D.

responsible for the rejection of primary and challenge infections in this host, and the expression of either or both responses may also be subject to individual variability. This also requires further study.

The wide variations in the responses of individual goats within and between treatment groups, especially in Experiment 2, were also evident in their egg counts, IgG and eosinophil counts and, to a lesser extent, in their pathological responses. Our study clearly showed that PCV and RBC count were reliable measures of host pathology and resilience to *H. contortus* infection in the WAD goat. Both parameters were also reliable indicators of intensity of infection with adult worms, but not of acquired immunity to the parasite. Body weight changes, on the other hand, were much less sensitive or reliable in these respects, which is not surprising since the infection in Experiment 2 was relatively mild in most animals and so its effect on body weight gain might not be easily detected over a relatively short period in the slow-growing WAD goat.

Overall, none of the other parameters namely, faecal egg counts, IgG level and circulating eosinophils was significantly associated with infection intensity or with any of the identified manifestations of acquired immunity, although such a relationship appeared to exist in some individual goats. For example, in group C, worm and egg count data for goat Nos 310 and 332 would seem to suggest that antibody and eosinophil responses are associated with intensity of infection. In the former goat, strong responses in the antibody response were associated with low worm burden (180) and faecal egg count (4 EPG) and in goat No. 332, weak responses were associated with high worm burden (1100) and faecal egg count (7250 EPG). However, this relationship did not hold consistently for other animals in this group. Furthermore, in group B, of the three animals, Nos 314, 317

and 321 which, on the basis of low worm burden and faecal egg counts (Fig 3), could be considered as relatively resistant, only No. 321 showed both eosinophil (Fig 8) and antibody (Fig 9) responses. Taken together, our observations on IgG and eosinophil responses seemed primarily to indicate exposure to infection rather than infection intensity or acquired immunity to infection. However, other workers have used both of these, and additional, parameters in the selection of parasite-resistant sheep (Woolaston et al 1996) and in *T. colubriformis*-infected sheep, eosinophil responses give an indication of the ability of the host to respond to challenge infection and to vaccination against the parasite (Dawkins et al 1989). Moreover, Douch et al (1995), have shown that parasite-specific serum IgG1 concentration in New Zealand Romney sheep infected with five species of GI nematodes, including *H. contortus* and *T. colubriformis*, is heritable and shows significant genetic correlation with production traits.

Of the above parameters the only one which is widely accepted and used as a criterion for the selection of parasite resistant sheep under field conditions is faecal egg counts. Numerous reports of studies in sheep exposed to naturally acquired or experimental infections with *H. contortus* have shown that the kind of variability in EPG which we observed in the WAD goat, is a characteristic feature of *H. contortus* and *T. colubriformis* infections in sheep and are indicative of genetic variation in immunological responsiveness to infection (Sreter et al 1994; Bisset et al 1996). Faecal egg counts are highly heritable in most of the sheep breeds which have been studied (Sreter et al 1994; Hohenhaus and Otteridge 1995; Bisset et al 1996) and its practical value has been demonstrated in a number of successful selective breeding programmes (Windon 1990; Gray 1991). There is good evidence that the same may be true of dairy goats in France (Richard et al 1990), fibre-producing goats of diverse genetic background in the Scottish Borders (Patterson et al 1996a, b) and the few tropical breeds of sheep and their crosses, which have been studied (Romjali et al 1996; Mugambi et al 1996; 1997). However, data from the even fewer reported studies of tropical goat breeds are less conclusive. Thus, although early experimental studies with *H. contortus* infections in Kenya (Preston and Allonby 1978) had shown that such variability exists, a more recent breeding study in Fiji did not find significant genetic variation or repeatability of FEC in mixed GI nematode infections (Woolaston et al 1992). This suggests the existence of breed and/or parasite strain-dependent differences in the responses of the different tropical goat breeds so far studied.

Although our data clearly show the existence of marked individual variations in host-acquired immune responsiveness in the goats studied, these are, by themselves, not direct evidence that the variations are genetic in origin. Equally, we cannot at present conclude that variation in acquired immune responsiveness is correlated with individual variations in EPG, IgG and eosinophil responses. Nevertheless, we believe that they are consistent with the suggestion that these patterns of responsiveness are an important feature of this host-parasite relationship and that FEC, in particular, is likely to be strongly influenced by host genetics in the WAD goat. These hypotheses can be easily tested experimentally.

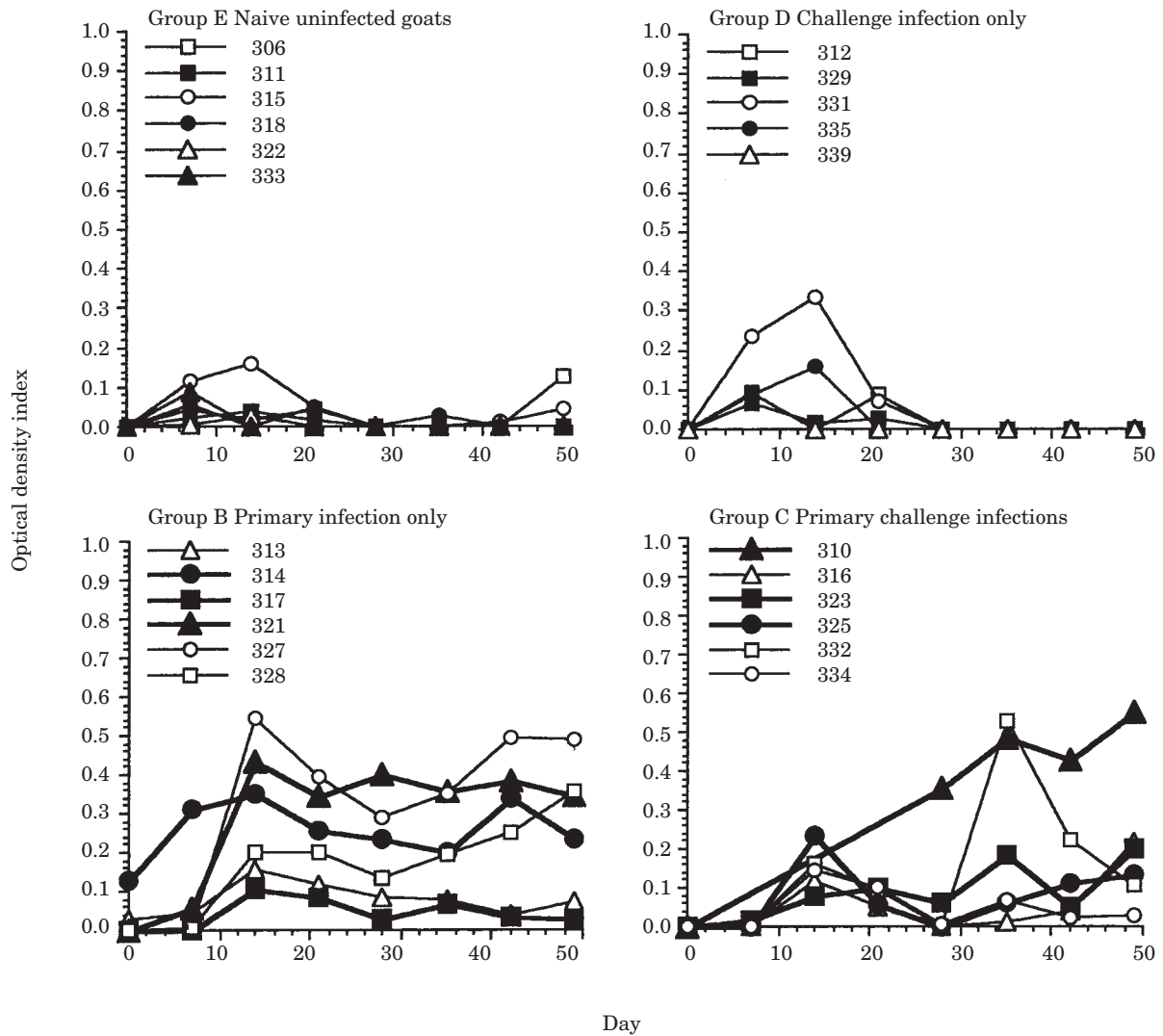


FIG 11: Experiment 2. Changes in the anti ES IgG antibody concentration in peripheral blood in individual animals from groups B, C, D and E throughout the experiment. The filled in symbols are animals identified earlier, on the basis of egg counts, as relatively resistant to infection and the open symbols are animals identified as susceptible to infection. See text and Fig 3 for full explanation and statistical analysis.

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