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The responses of a tropical breed of domestic rabbit, *Oryctolagus cuniculus*, to experimental infection with *Trichostrongylus colubriformis*

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Abstract

Clinical, parasitological and pathological responses of a tropical out-bred domestic rabbit to experimental *Trichostrongylus colubriformis* infection were used to evaluate its suitability as a laboratory host and model for studying the host–parasite relationships of *T. colubriformis*. In the first experiment, three groups each of 16, predominantly juvenile male, 8- to 10-week-old rabbits were given a single pulse infection with 500, 5000 or 25000 infective larvae (L3) of *T. colubriformis*, to represent low, medium and high levels of infection, respectively. A fourth group of 16 rabbits of similar age formed the uninfected controls. In the second experiment, two groups of 10 juvenile (8- to 10-week-old) and 10 adult (8- to 10-month-old) rabbits were similarly infected with 20000 L3, with appropriate naïve controls. Prepatency was 14 and 16 days and peak faecal egg counts occurred on days 24 and 20 after infection in young and adult rabbits respectively. Peak worm counts occurred on day 14 in both age groups and at all levels of infection. Subsequently, parasite burdens declined in a highly significantly dose- and age-dependent manner. At low and moderate levels of infection, approximately 83–98% of worms were recovered from the first 60 cm of the small intestine. Worm fecundity was also significantly influenced by host age and larval dose. Host age also had a significant effect on worm length. Infections with *T. colubriformis* were associated with a highly significant loss of body weight, accompanied by anorexia, diarrhoea and 25% mortality at high dose levels during the patent period of infection. There were no significant changes in packed cell volume and eosinophil counts at all ages and levels of infection but significant lymphocytosis occurred at the high dose level between days 7 and 21. Parasite-specific serum IgG responses were not related to worm burden. Overall, data showed that this miniature, docile and relatively inexpensive breed of rabbit is a potentially valuable laboratory host for studying *T. colubriformis* infections. The larval dose, duration of infection and host age were major determinants of host responsiveness to primary infections in this rabbit genotype.

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Introduction

Trichostrongylus colubriformis and *Haemonchus contortus* are two of the most prevalent and economically important gastrointestinal (GI) nematode parasites of small ruminants (sheep and goats) in the humid and sub-humid tropics (Chiejina, 1994; Baker *et al.*, 1998). In the sub-humid zone of Nigeria, both nematode species are invariably involved in documented outbreaks of parasitic gastroenteritis (PGE) in small ruminants, causing considerable, but as yet unquantified, economic losses through mortality, poor carcase quality and impaired reproductive performance. Consequently, the nematodes are currently a major constraint in attempts to improve the productivity of the West African Dwarf (WAD) sheep and goat through intensive rearing on pasture in the subhumid zone of Nigeria (Chiejina, 1987). Despite the importance of these strongylid infections, only relatively few controlled studies have been carried out on their epidemiology (Chiejina & Fakae, 1984, 1989; Fakae, 1990) and none on their pathogenesis, pathophysiology, host acquired and genetic resistance in WAD breeds.

Recently, we conducted a number of studies to evaluate the suitability of a local breed of domestic rabbit as a laboratory host of *T. colubriformis* and a relatively inexpensive laboratory model for a preliminary study on aspects of the pathogenesis and immunology of this infection in the WAD sheep and goat. Other breeds of rabbit, notably the New Zealand White (NZW), which are not readily available in Nigeria, have been used successfully for similar purposes in other countries (Purvis & Sewell, 1972; Barker, 1975; Wedrychowicz & Bezubik, 1988, 1990). Indeed, laboratory animal model systems, in general, have contributed substantially to our understanding of host–parasite relationships in single and concurrent infections in man and his domesticated animals (Darji, 1982; Christensen *et al.*, 1987; Sileghem *et al.*, 1994; Onah & Wakelin, 1999). The present paper employs some parameters of the parasitological, pathological and humoral immune responsiveness of this tropical breed of rabbit to primary infections with *T. colubriformis*, to evaluate its suitability as a laboratory animal model for fundamental studies on the host–parasite relationship of *T. colubriformis*. This nematode is the only species among the two major GI trichostrongylids of goats in the humid tropics that occurs naturally in the domestic rabbit (Samson, 1970).

Materials and methods

Experimental design

Two experiments were carried out. Experiment 1 examined the effect of larval dose on host responses in juvenile (8- to 10-week-old) rabbits to infection, to determine a working infective dose for future experiments. Experiment 2 examined the effect of host age on responses to infection, using juvenile and adult (6- to 8-month-old) rabbits. Details of the experimental groups and larval dosage are given in table 1.

Rabbits and their management

The breed of rabbit used in this study is commonly found in the Nsukka area of eastern Nigeria, where it is kept mainly for meat. Little is known about its origin and breed characteristics and there are no records of this rabbit having previously been used for parasitological research. Available records indicate that when full-grown, it can attain a maximum size of only 2.0 kg. This rabbit, therefore, is much smaller than the better known European or temperate breeds such as the NZW, California and Chinchilla giant with equivalent body weights of 4.1–5.4 kg, 3.6–4.8 kg and 5.4–7.3 kg, respectively (Arrington & Kelly, 1976).

Fifty six male and eight female rabbits were purchased at 6–8 weeks of age from local producers and used at 8–10 weeks of age. However, in experiment 2, adult rabbits born and reared in our laboratory and comprising 24 males and six females were used. All rabbits were weighed and individually identified with numbered neck bands. Blood and freshly voided faeces were collected for routine laboratory analyses for evidence of haemoprotozoans, especially *Trypanosoma* species, and for helminth ova and coccidia oocysts respectively. All rabbits were subsequently dosed orally with 10 mg kg^{-1} body weight of fenbendazole (Panacur®, Hoechst, Nigeria Ltd), via a syringe and stomach tube. Rabbits were also dusted with Asuntol® powder (Coumaphos®, Bayer, Nigeria, Ltd) against ectoparasites, and treated with a coccidiostat containing furazolidone and sulphadimidine (Francocine®, SAL Veterinary Services, Nigeria, Ltd) in drinking water for 7 days, as recommended by the manufacturers.

The rabbits were then housed in pairs in 1.0 m × 1.0 m × 0.5 m metal cages with wire mesh floors supported by metal grids and raised 0.5 m off the floor in

Table 1. Experimental design.

Experiment number and group ()	Number of larvae (L3) administered on D0	Number of rabbits per group	Age of rabbits (weeks)	Number of rabbits killed per necropsy day	Necropsy days
1 (A)	500	16	8–10	4	7, 14, 21 and 28
1 (B)	5000	16	8–10	4	7, 14, 21 and 28
1 (C)	25000	16	8–10	4	7, 14, 21 and 28
1 (D)	Uninfected	16	8–10	4	7, 14, 21 and 28
2 (E)	20000	10	8–10	5	14 and 28
2 (F)	Uninfected	5	8–10	5	28
2 (G)	20000	10	24–32	5	14 and 28
2 (H)	Uninfected	5	24–32	5	28

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concrete pens. Green forage, collected from experimental paddocks, inaccessible to livestock, were liberally soaked and rinsed in water, drip-dried and fed *ad libitum* to the rabbits. This was supplemented with a modified proprietary poultry feed containing palm kernel cake and rice bran (Animal Care Services Consult, Nigeria Ltd). Drinking water was provided *ad libitum*. Faeces were collected in galvanised metal trays and disposed of and the trays cleaned every morning.

Parasitological procedures

Trichostrongylus colubriformis *infection*

Infective larvae (L3) of *T. colubriformis* were obtained from donor rabbits infected with a sheep strain of the parasite and used within 14 days of harvesting from faecal cultures. Estimated doses of L3 (table 1) were administered via a stomach tube.

Faecal egg counts

Faecal egg counts (FEC) were carried out using the salt flotation and modified McMaster techniques, as appropriate (MAFF, 1977). Counts were done on arrival at the laboratory, the day of initial infection (D0) and every two days, thereafter.

Worm counts and measurements

Rabbits were killed for necropsy as necessary (table 1). Each rabbit was carefully restrained and humanely killed. After death of the animal, the small intestine (SI) was quickly removed, divided with double ligatures into three portions, namely, the first and second 30 cm lengths and the rest of the SI. Each portion was transferred to a Petri dish and processed for worm counts (MAFF, 1977). The large intestines of the rabbits were also processed for worm counts. A minimum of 13–20 adult worms of each sex were randomly removed for determination of worm length (experiment 2), male to female ratio (MFR) and fecundity of female worms (experiments 1 and 2). The fecundity of female worms was assessed from the number of fully developed ova in the uterus of each worm, expressed as eggs per female (EPF). Worm measurements were determined from camera lucida drawings on calibrated paper, using a map measure (Fakae *et al.*, 1994).

Haematology and body weight

Packed cell volume (PCV), total and differential white blood cell (WBC) counts were carried out weekly between D0 and day 28, on fresh blood collected from the ear vein in bottles containing the anticoagulant, EDTA, according to Dacie & Lewis (1995). However, in experiment 2, examinations were made on D0, D14 and D28 only. Body weights were also measured weekly. Data were expressed as percentage changes from initial (D0) values.

Serum IgG

Parasite-specific serum IgG levels were measured weekly in experiment 2 using a standard ELISA technique (Chieng, 1987) on previously determined optimum L3 somatic antigen concentrations, serum and IgG-enzyme (alkaline phosphatase) conjugate dilutions of 5 μg ml^{-1}, 1:25 and 1:1000, respectively. Known naïve and positive (hyperimmune) sera were included in every test plate. Absorbance values were measured as optical density (OD) at 405 nm, using a Dynatech MR 200 reader. Corrected OD values were calculated according to Fakae *et al.* (1994).

Statistical analysis

Data on worm establishment in experiment 1, as a percentage of larval dose, and worm distribution (worm recovery according to segment of the SI), as a percentage of the total worm burden, were normalized by angular transformation and analysed by two-way analysis of variance (ANOVA) (Fowler & Cohen, 1990), with larval dose (3 levels, corresponding to 500, 5000 and 25000 L3) and duration of infection (4 levels, corresponding to days 7, 14, 21 and 28 after infection) as fixed points. In experiment 2 worm counts on D14 and D28 were analysed by two-way ANOVA with age of rabbits (2 levels: young and adult) and duration of infection (2 levels: D14 and D28) as fixed factors. Worm lengths on D14 and D28 were compared using a completely randomized design ANOVA (Fowler & Cohen, 1990) with rabbit age (2 levels) as a fixed factor. Data on eggs per female worm (EPF) were also examined using two-way ANOVA with dose and duration (each at 3 levels) as fixed points. Data on MFR were analysed by three-way ANOVA with dose (3 levels), duration (4 levels) and rabbits (4 levels) as fixed points. Serum IgG levels, changes in body weight, PCV, lymphocyte, eosinophil and total WBC counts were analysed using repeated measures ANOVA, with Instat (GraphPAD software Inc., USA). In all cases, values of $P < 0.05$ were considered significant.

Results

Experiment 1

Worm establishment and duration of infection

Worm counts at all doses of L3 were characterized by marked individual variability, particularly in the high and low infection groups (table 2). Peak counts were observed on D14 and this was followed by a rapid, dose-dependent rejection of the infection, so that relatively few worms survived to D28 in both groups. In contrast, the small peak in worm burden on D14 in the low infection group was maintained until the end of the experiment, with little loss of worms (fig. 1).

There was a significant effect of larval dose on the percentage worm establishment ($F_{2,36} = 13.511$, $P < 0.001$), with the highest and lowest percentages (mean 34.30% and 19.0%, respectively) occurring in the high and low dose groups respectively. Similarly, a significant effect on the duration of infection ($F_{3,36} = 4.653$, $P < 0.01$) was apparent in the high dose group, but there was no significant interaction between dose and duration of infection ($F_{6,36} = 1.816$, $P > 0.05$). No worms were recovered from control rabbits.

At the low (group A) and medium (group B) infection levels, worms were located primarily in the first two

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Table 2. Mean ± SE and range ( ) in the number of *Trichostrongylus colubriformis* in rabbits on days 7, 14, 21 and 28 post-infection (experiment 1).

Experimental group and larval dose	Day 7	Day 14	Day 21	Day 28
Group A	161.5 ± 69.8	170.8 ± 40.8	165.3 ± 10.1	151.0 ± 0.09
Low level infection, 500 L3	(44–360)	(98–270)	(144–192)	(130–192)
Group B	676.3 ± 219.4	1415.0 ± 70.7	1181.3 ± 24.2	721.3 ± 175.5
Medium level infection, 5000 L3	(540–1370)	(1310–11615)	(420–1575)	(235–1020)
Group C	4957.5 ± 911.8	5970 ± 1050.0	2894 ± 1621.0	214.8 ± 135.0
High level infection, 25000 L3	(2515–6934)	(4006–8770)	(185–6459)	(4–600)

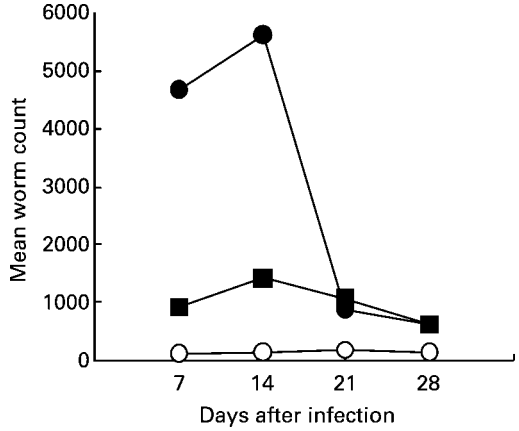

Fig. 1. Geometric mean worm counts juvenile rabbits infected with 500 (○), 5000 (■) or 25000 (●) larvae of *Trichostrongylus colubriformis* up to 28 days after infection.

30 cm sections of the SI throughout the duration of the experiment (fig. 2a,b). Both sites accounted for approximately 83–98% of the total worm burden in these two groups. However, in the high infection group there was marked distal movement of worms to the last segment of the SI, particularly on D14 and D21 (fig. 2c), with nearly 70% of the worm burden located in the distal small intestine on D14 and a return, on D28, to the normal pattern seen in groups A and B. ANOVA of the effects of larval dose and duration of infection on worm recoveries from each segment of the SI, showed that the main effect of larval dose was highly significant in segments 1 and 3 ($F_{2,36} = 17.879$, $P < 0.001$ and $F_{2,36} = 19.694$, $P < 0.001$ respectively) but not in segment 2 ($F_{2,36} = 0.414$, $P > 0.05$). The effect of the duration of infection was significant in the three segments.($F_{3,36} = 2.984$, $P < 0.05$, $F_{3,36} = 8.258$, $P < 0.001$, and $F_{3,36} = 3.998$, $P < 0.05$, respectively). The interaction between the two factors was also significant in all segments ($F_{6,36} = 2.558$, $P < 0.05$, $F_{6,36} = 3.983$, $P < 0.01$ and $F_{6,36} = 5.775$, $P < 0.01$ respectively)

There were significant dose-dependent differences in the mean MFR and EPF (fig. 3a,b) between infected groups. Larval dose and the duration of infection had highly significant effects on MFR ($F_{2,18} = 278.309$, $P < 0.001$ and $F_{3,18} = 226.769$, $P < 0.001$, respectively). The rabbit, as a factor, had no significant effect ($F_{3,18} = 2.253$, $P > 0.05$), indicating homogeneity of the host population. The dose and duration of infection also had highly significant effects on EPF ($F_{2,342} = 77.427$,

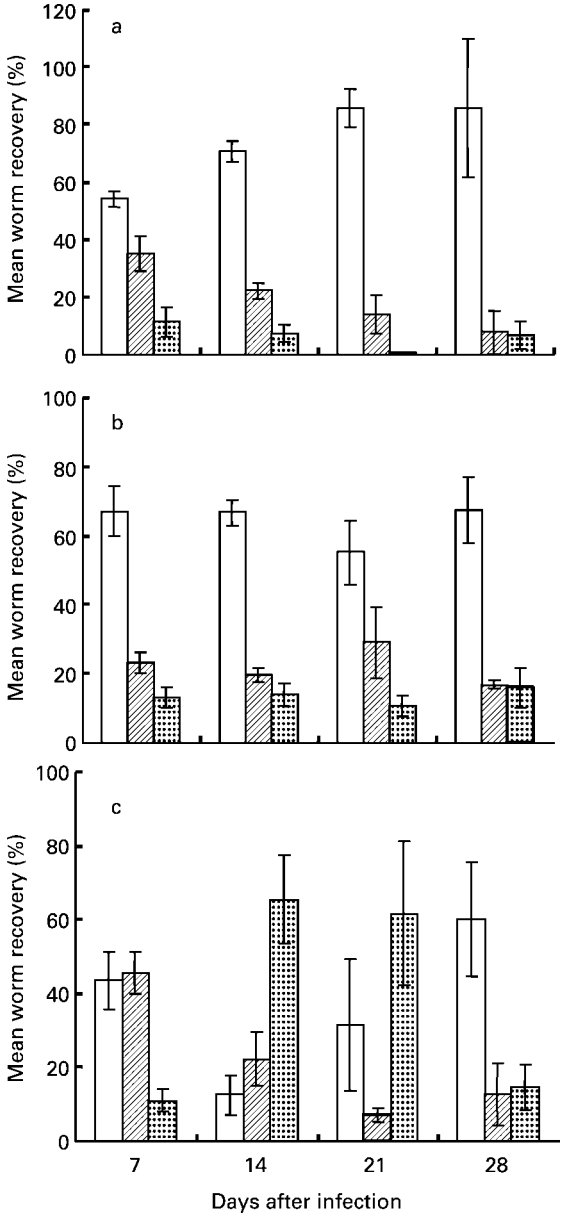

Fig. 2. The distribution of *Trichostrongylus colubriformis* expressed as a percentage of worms recovered (±SE) from the small intestine (SI) of juvenile rabbits infected with either (a) 500 or (b) 5000 or (c) 25000 larvae up to 28 days after infection. (□, First 30 cm of SI; ▨, second 30 cm of SI; ▩, remainder of SI.

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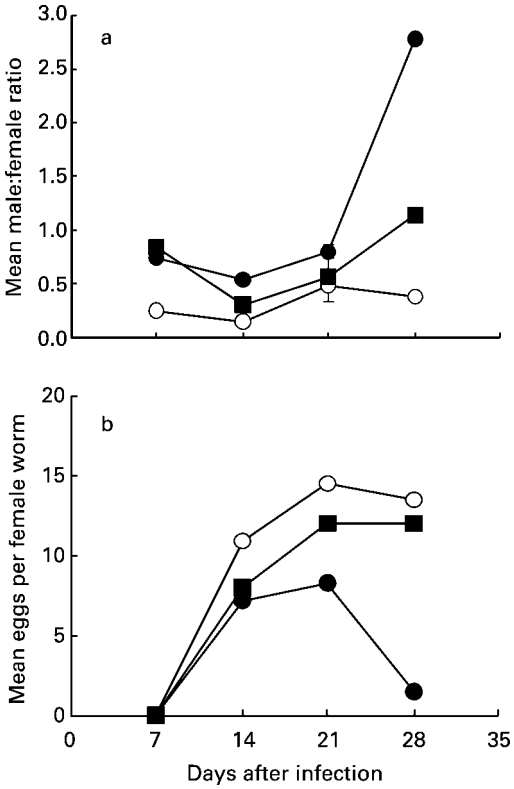

Fig. 3. The mean ratio (±SE) of (a) male to female worms and (b) the mean number of eggs per female worm recovered from juvenile rabbits infected with 500 (○), 5000 (■) or 25000 (●) larvae of *Trichostrongylus colubriformis* up to 28 days after infection.

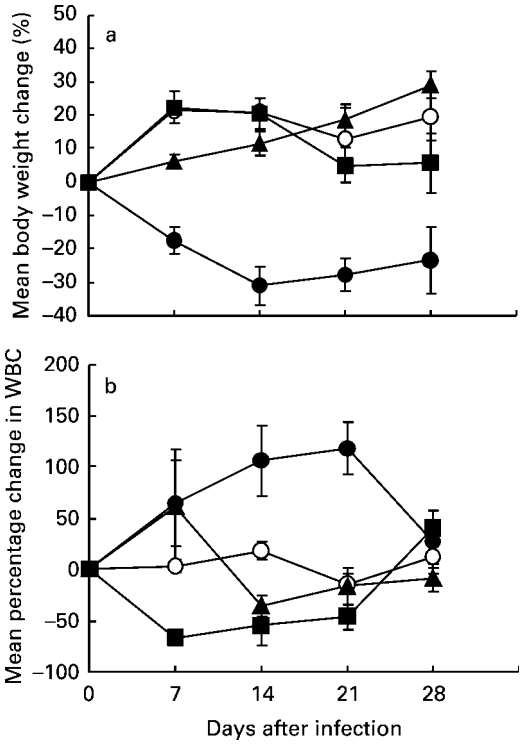

Fig. 4. The mean percentage change in (a) body weight and (b) mean white blood cell count (±SE), of 8–10 week-old rabbits infected with 500 (○), 5000 (■) or 25000 (●) infective larvae of *Trichostrongylus colubriformis*. ▲, Control.

$P < 0.001$ and $F_{2,342} = 13.752$, $P < 0.001$, respectively), with highly significant interactions occurring between them ($F_{4,342} = 13.464$, $P < 0.001$).

Female worms predominated between D7 and D21 in groups B and C and to D28 in group A but a marked reversal of MFR in favour of the male worms occurred in groups B and C between D21 and 28 (fig. 3a). Thus, progressively more female worms were lost and, in group C, only 26.89% of the worms recovered from all rabbits on D28 were females, with a markedly reduced fecundity (fig. 3b).

Pathology

Infection was associated with highly significant ($F_{3,12} = 10.034$, $P = 0.001$) dose-dependent effect on body weight. Severe weight loss in rabbits in group C (fig. 4a) was associated with anorexia, varying degrees of emaciation and diarrhoea, particularly from D14.

There were no significant differences in PCV between groups A, B and C ($F_{3,9} = 0.755$, $P = 0.547$). In contrast, the medium (group B) and high (group C) levels of infection resulted in leucopaenia and leucocytosis respectively, between D7 and D21, with return to normal values on D28 (fig. 4b). Leucocytosis was due primarily to corresponding change in lymphocyte counts (figs 4b, 5a). Transient but pronounced eosinophilia occurred in group C (fig. 5b) but the overall effect on eosinophil counts was not significant ($F_{3,16} = 2.372$, $P > 0.05$). The effects of treatments were significant only for changes in total WBC ($F_{3,12} = 3.562$, $P < 0.05$) and lymphocyte counts ($F_{3,12} = 5.280$, $P = 0.015$).

Experiment 2

Worm establishment and duration of infection

The pre-patent period was longer by two days in adult than in juvenile rabbits and the subsequent sharp rise in FEC (fig. 6) was more pronounced and sustained in juvenile than in adult rabbits. Worm counts were made only on D14 and D28, as it was found in experiment 1 that rapid worm rejection occurred mainly during this period. This observation was confirmed by data for juvenile rabbits (fig. 7), which were also more susceptible, and harboured heavier worm burdens than the adults on both D14 and D28 (fig. 7). The main effects of host age and duration of infection were highly significant ($F_{1,16} = 32.530$, $P < 0.001$ and $F_{1,16} = 26.260$, $P < 0.001$, respectively). The interaction between host age and duration of infection was also highly significant ($F_{2,16} = 16.135$, $P < 0.001$).

There was no significant difference in MFR in juvenile and adult rabbits on D14 an D28 but male and female worms recovered from adult rabbits were significantly shorter than corresponding sexes from juvenile rabbits (D14 males and females: $F_{1,24} = 8.448$, $P < 0.01$ and $F_{1,24} = 7.451$, $P < 0.05$, respectively; D28 males and

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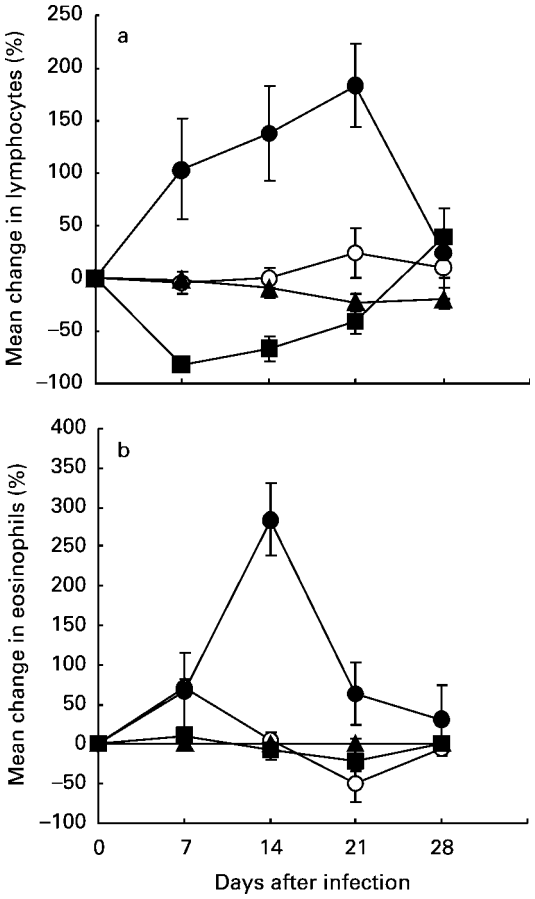

Fig. 5. The mean percentage change in (a) circulating lymphocyte and (b) eosinophil counts of juvenile rabbits infected with 500 (○), 5000 (■) or 25000 (●) larvae of *Trichostrongylus colubriformis* up to 28 days after infection. ▲, Control.

females: $F_{1,24} = 11.914$, $P < 0.01$ and $F_{1,24} = 4.885$, $P < 0.01$, respectively). For example, the mean length of male worms from the young and adult rabbits on D28 were 4.05 ± 0.01 and 3.59 ± 0.09 mm and the corresponding values for female worms were 4.98 ± 0.15 and 4.29 ± 0.17 mm, respectively.

Serum IgG

Host age significantly influenced IgG responses ($F_{2,8} = 8.879$, $P < 0.01$). Infected juvenile rabbits showed weak parasite-specific serum IgG responses, even during and following the rapid worm rejection phase. This contrasted with the relatively strong responses in adult rabbits, particularly on D14 (fig. 8).

Discussion

The domestic rabbit is probably the only conventional laboratory animal that is not only a natural host for the strain of *T. colubriformis* (Skidmore, 1932; Dickmans, 1937; Samson, 1970) but is also susceptible to the ruminant strain of this nematode (Soulsby, 1982). Consequently, the

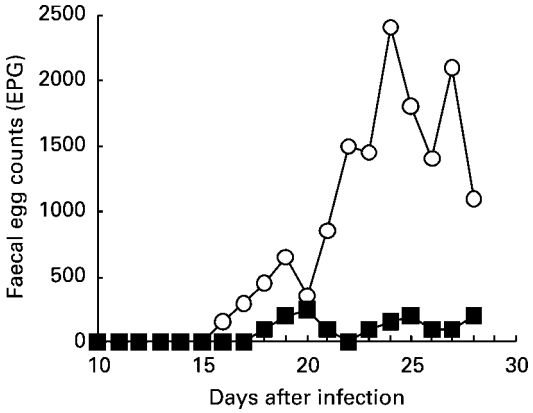

Fig. 6. Faecal egg counts of groups of juvenile (○) and adult (■) rabbits infected with 20000 larvae of *Trichostrongylus colubriformis* up to 28 days after infection.

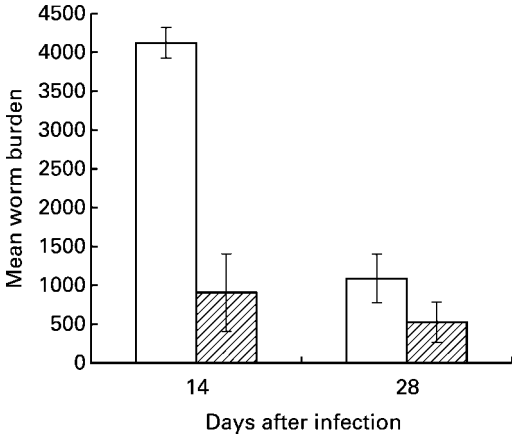

Fig. 7. Mean worm burdens (± SE) of juvenile (□) and adult (▨) rabbits infected with 20000 larvae of *Trichostrongylus colubriformis* up to 28 days after infection.

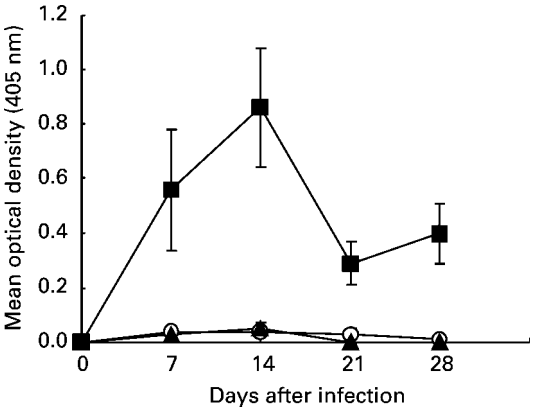

Fig. 8. Mean serum IgG levels (expressed as mean optical density ± SE) in juvenile (○) and adult (■) rabbits infected with 20000 larvae of *Trichostrongylus colubriformis* up to 28 days after infection. ▲, Control.

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NZW and other popular breeds of rabbit have been successfully used for studies on experimental infections (Purvis & Sewell, 1972; Barker & Ford, 1975; Wedrychowicz & Bezubik, 1988, 1990; Wedrychowicz *et al.*, 1989).

There are no published data on natural or experimental *T. colubriformis* infections in the tropical breed of rabbit used in the present work. Nevertheless, the present data suggest that this miniature breed is also a suitable laboratory host for this nematode species. The small size and docile temperament of this breed of rabbit made it easy to handle and, more importantly, it was found to be readily susceptible to the sheep strain of *T. colubriformis*, with a slightly shorter pre-patent period (14–16, instead of 20 days) to that of infections in small ruminants (Soulsby, 1982). The tropical breed of rabbit was also capable, even at 8–10 weeks of age, of controlling primary infections with doses of up to 25000 L3. However, in this juvenile age group, the kinetics of worm loss was parasite dose- and host age-dependent. The older the host and the higher the larval dose, the more rapid was the rate of worm loss, with nearly a complete loss in 50% of rabbits in group C. In experiment 1, the duration of infection profoundly influenced worm establishment but there was no interaction between the dose and duration of infection. In experiment 2, on the other hand, not only did the duration of infection influence worm rejection but it also interacted strongly with host age in determining the worm rejection phenotype of the rabbits.

The failure of group A rabbits to reject a significant part of established (D14) worm burden suggests that a threshold is necessary to trigger worm rejection. Such a threshold must have been reached in group B and possibly exceeded in group C.

The distribution of worms along the SI is similar to that in small ruminants and in the larger and widely studied, 'exotic' breeds of rabbit, with a distinct preference for the proximal region of the gut (Barker, 1975; Barker & Ford, 1975; Soulsby, 1982). The exception was during the rapid expulsion phase (D14–D21) in group C rabbits, in which there was a distinct aggregation of worms towards the distal region of the SI, which is consistent with the accelerated decline in worm burden in this group during this period. Analyses of worm recoveries, in each segment of the gut, showed that worm numbers were clearly influenced by dose and duration of infection.

It is noteworthy that the steep rise in FEC in juvenile rabbits between D20 and D24 and peak counts in juvenile and adult rabbits on D24 and D20 respectively, occurred when the rapid rejection of worms, which normally commenced from D14, was well advanced. Chiejina & Sewell (1974) observed a similar lack of synchrony between egg counts and worm rejection in young lambs and suggested that worm rejection and impaired fecundity, generally associated with host acquired immunity in sheep, might be caused by different host effector mechanisms. However, in ovine trichostrongylosis, worm rejection was usually preceded by the inhibition of ovulation.

The weak IgG response in group E is probably indicative of the well known poor relationship between this immune response and worm rejection (Schallig & van Leeuwen, 1977; Schallig, 2000). The use of L3 antigen might also influence this response, since the specificity of host immune response to *T. colubriformis* antigens in sheep and rabbits appears to be parasite stage-specific (Wedrychowicz *et al.*, 1989; Wedrychowicz & Bezubik, 1990; Griffiths & Pritchard, 1994). On the other hand it is possible that other immune mechanisms not examined in this study contributed to the very rapid worm rejection observed in juvenile rabbits. The present study, therefore, does not exclude a role for host acquired immunity in determining the worm rejection phenotype observed in the juvenile rabbit.

The precise mechanisms of acquired immunity to *H. contortus* and *T. colubriformis* infections in ruminants are unknown. In general, the humoral immune response is a poor correlate of protective immunity in GI nematode infections of sheep (Schallig & van Leeuwen, 1997), as in the rabbit, although increased concentrations of parasite-specific IgG, IgG1, IgG2 and IgA may be present in the gut mucosa and mucus in *T. colubriformis* and *H. contortus*-infected sheep (McClure *et al.*, 1992; Gill *et al.*, 1994). Data from a recent study by Gill *et al.* (2000) has implicated a strong Th2-type response in immunity to *H. contortus* in genetically resistant sheep. Young lambs appear to be incapable of mounting a strong Th2 mediated response to these nematodes (Schallig, 2000). It has also been suggested that in vaccinated sheep and goats, CD4 + T lymphocytes appear to work synergistically with antibodies to confer protection (Karanu *et al.*, 1997). The domestic rabbit–*T. colubriformis* model could be a suitable system with which to further investigate these mechanisms of acquired immunity to GI nematode infections of small ruminants.

It is generally assumed, especially in ruminants, that the regulation of the population and fecundity of GI nematodes is mediated primarily by host immune mechanisms. However, Stear *et al.* (1999) have pointed out that in *Ostertagia circumcincta*-infected sheep, density-dependent mechanisms associated with overcrowding of worms in the abomasum and host genetic effects on worm length have major influences on the intensity of infection and worm fecundity, respectively. In this host–parasite relationship, worm fecundity, and hence FEC, apart from being directly related to the length of female worms, decrease as the worm burden increases. Therefore, the influence of host genetics on worm length is considered to be more important than its effect on worm numbers in determining the egg count response phenotype of individual lambs.

The present data suggest that overcrowding, the duration of infection and host age play major roles in determining parasite burdens and fecundity in the rabbits. No information is available on genetic influences on these parameters. In groups C and E in particular, overcrowding of worms must have occurred at these large dosage levels, given the relatively short length of the small intestine of juvenile rabbits (mean length of small intestine, $\pm$SD = 146.80 $\pm$ 0 3.0 cm, n = 48). This is the most likely explanation for the extremely rapid worm rejection at the high dose levels in this particular age of rabbit, although host age and acquired immunity might also have been contributory factors. In experiment 2 on the other hand, the magnitude of the peak and the rate of decline in worm counts were essentially host age-dependent, with substantially larger burdens and a

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more rapid worm loss occurring in juvenile than in adult rabbits.

A small peak in worm burden in adult rabbits is partly an age-related adverse effect on larval development and worm establishment and partly an immunologically-mediated early worm loss. If host acquired immunity played any major role in worm rejection between D14 and D28 in experiment 2, it must have occurred in the juvenile rather than in the adult rabbits. This would be at variance with the situation in young lambs and kids (Manton *et al.*, 1962; Chiejina & Sewell, 1974; Schallig, 2000).

Clinical and haematological responses were also generally consistent with those commonly found in experimental and naturally-occurring trichostrongylosis of small ruminants (Horak *et al.*, 1968; Chiejina & Sewell, 1974; Soulsby, 1982), especially with regard to lymphocytosis, symptoms of anorexia, diarrhoea and weight loss in clinically affected rabbits and the absence of anaemia. However, eosinophilia was an inconsistent finding and bore no relationship to worm number and fecundity. This is also true of infections in goats (Fakae *et al.*, 1998). In sheep, peripheral eosinophilia, which occurs in GI nematode infections of genetically resistant animals, are sometimes useful phenotypic markers of genetic resistance to GI nematodes (Woolaston *et al.*, 1996; Hohenhaus *et al.*, 1998).

Overall, the present experiments have demonstrated that this miniature, tropical breed of rabbit is a potentially valuable laboratory host and model for the study of host–parasite relationships in *T. colubriformis* infections. Larval dose, the duration of infection, density-dependent influences resulting from large doses of infection and host age were found to be the major determinants of intensity of infection, survival and fecundity in this genotype of rabbit.

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