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Heligmosomoides polygyrus and *Trypanosoma congolense infections* in mice: effect of immunisation by abbreviated larval infection

B.B. Fakae [a,*], L.J.S. Harrison [b], C.A. Ross [b], M.M.H. Sewell [b]

[a] *Department of Veterinary Parasitology and Entomology, University of Nigeria, Nsukka, Nigeria*
[b] *Centre for Tropical Veterinary Medicine, Easter Bush, ROSLIN, Midlothian EH25 9RG, Scotland, UK*

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Abstract

Concurrent African trypanosome and gastrointestinal helminth infections are prevalent in sub-humid savannah where they are endemic. However, acquired resistance in animals varies with their responder status and exposure. As a guide to study in the definitive hosts, the effects of *Trypanosoma congolense* infection on the development and maintenance of homologous *Heligmosomoides polygyrus* resistance were investigated in outbred TO mice. These mice were immunised by abbreviation of larval infection. Immune or naive mice were either infected with 500 infective larvae (L_3) of *H. polygyrus* and/or 10^4 bloodstream forms of *T. congolense* or were not infected. The outcome of infection was monitored by routine parasitological and immunological techniques for 30 days after the day of the *T. congolense* infection. Significantly more immune mice concurrently infected with both parasites survived than did immune mice in which *H. polygyrus* was superimposed on a 10-day-old *T. congolense* infection. Although all the mice in this latter group died before the end of the experiment, larval immunisation prolonged their survival, relative to similarly treated naive mice. The antibody titres to *H. polygyrus* in the sera of immune mice challenged with *H. polygyrus* alone were significantly higher than those of immune mice concurrently infected with both parasites but the levels of protection obtained were comparable. It is concluded that *T. congolense* may not completely block the strong acquired resistance induced by abbreviated *H. polygyrus* larval infection in TO mice but is capable of interfering with protective responses, especially if the trypanosome infection occurs prior to *H. polygyrus* challenge infection.

Keywords: *Heligmosomoides polygyrus*; Larval infection; *Trypanosoma congolense*; Concurrent infection; Mouse model; Immunisation; Immunosuppression.

* Corresponding author.

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1. Introduction

Gastrointestinal nematodes are recognised as a major cause of impaired productivity in ruminants in the tropics (Chiejina, 1986; Fabiyi, 1987). These infections are mostly subclinical (Chiejina, 1987; Fakae, 1990) probably due to acquired or innate resistance. In a recent study, it was possible to segregate West African Dwarf goats into categories showing either high or low responses to challenge *Haemonchus contortus* infection (Fakae et al., 1999). In natural populations of ruminants in sub-Saharan Africa, gastrointestinal nematode and African trypanosome infections are endemic (Fakae and Chiejina, 1993). Trypanosomes are known to cause non-specific depression of immune responses to a variety of heterologous antigens (Murray et al., 1980; Wakelin, 1984). *Heligmosomoides polygyrus* and *Trypanosoma congolense* infection in mice has been validated as a cheap and suitable laboratory model for studying interactions between trichostrongyles and African trypanosome infections (Fakae et al., 1994). A recent report demonstrated that mice immunised by termination of adult *H. polygyrus* infection lost almost all the protection acquired against a homologous challenge during a concurrent infection with *T. congolense* (Fakae et al., 1997). It is not yet understood whether this complete loss of protection was due to a total blockage of immune effector mechanisms by the protozoa or due to an initial inferior protective response mounted by the mice. If immune blockage occurs, then the suggested genetic and immunological control of nematodosis (Gray, 1997; Klei, 1997) in trypanosome endemic areas may be almost unattainable.

In contrast to immunisation by treatment of an already existing adult *H. polygyrus* burden, abbreviation of larval infections (while still in the histotrophic phase) have been shown to protect mice completely from homologous challenge (Wahid and Behnke, 1992; Fakae, 1993). The main purpose of the experiments described in this paper was to determine the effects of *T. congolense* infection on the development and maintenance of the almost solid homologous *H. polygyrus* immunity induced in mice by exposure to an abbreviated larval infection. In addition, the effects of trypanosome infection either concurrently or before the challenge with *H. polygyrus* infection were compared, to elucidate the extent to which an earlier trypanosome infection compromised the animals.

2. Materials and methods

2.1. Experimental animals and parasites

Six to eight week old outbred female TO mice raised as specific pathogen free and weighing 20–25 g were used for the study. The third larval stage (L3) of the nematode, *H. polygyrus*, were raised in vermiculite cultures. *T. Congolense* (TREU 1881) was obtained from a liquid nitrogen stabilate bank of blood stream forms and mice were infected intraperitoneally. The origin and maintenance of these parasites have been described in detail by Fakae et al. (1994).

2.2. Immunisation of mice

Mice were immunised by oral infection with 500 *H. polygyrus* L3 and then, 6 days later, the infection was terminated by oral administration of ivermectin (Eqvalan paste, MSD-

Table 1
Experimental design: six groups of mice were pre-exposed by oral infection with 500L3 of *Heligmosomoides polygyrus* and drug treated 6 days later to eliminate the larval infection. Another three groups of mice were simply given drug treatment. Twenty-one days after the drug treatment (Day 0) the various groups of mice were challenged with 500 L3 *H. polygyrus* (HP) and/or 10^4 *T. congolense* (TC).

Group	Immunising infection	Anthelmintic treatment	Challenge infection	
	(Day 27)	(Day 21)	(Day 0)	(Day 10)
(A) Immune concurrent	+	+	HP + TC	–
(B) Immune nematode	+	+	HP	–
(C) Immune trypanosome	+	+	TC	–
(D) Immune split challenge	+	+	TC	HP
(E) Immune Nematode	+	+	–	HP
(F) Immune control	+	+	–	–
(G) Naïve nematode	–	+	HP	–
(H) Naïve split controls	–	+	TC	HP
(I) Naïve control	–	+	–	–

AGVET), at the rate of 20 mg/kg body weight. The drug was diluted in distilled water to adjust the dose to 20 mg/kg body weight, administered in a volume not exceeding 0.1 ml.

2.3. Experimental design

Mice were randomly allotted into one group of five mice and nine groups of eight mice. The group of five mice was killed at the beginning of the experiment in order to determine the mean carcass and eviscerated carcass weights, the other mice being treated according to the experimental design outlined in Table 1. For the purposes of the study the six groups (A, B, C, D, E and F) were immunised and referred to as immune mice. Three groups designated naive mice (G, H and I) were not infected, but were treated with the anthelmintic as the others and then used as parasite viability controls as detailed in Table 1. The dose rate of anthelmintic employed has been demonstrated to be effective in removing all larval *H. polygyrus* from the intestinal wall of mice (Wahid and Behnke, 1992) and this was confirmed in experiments conducted prior to this study (Fakae, 1993). The results are discussed relative to the first day of the challenge infection, which was 21 days after the termination of the primary infection and is referred to as Day 0.

Three possible additional controls were omitted on ethical grounds and to reduce the use of mice. These controls were all naive mice groups and would have consisted of mice given a concurrent infection on Day 0, mice infected with the trypanosome alone on Day 0 and mice infected with the nematode alone on Day 10.

2.4. Monitoring

Mice were bled from the tail daily for the estimation of parasitaemia (Herbert and Lumsden, 1976) and weekly for the determination of packed cell volume (PCV) and for serum collection. Faecal egg count was performed on pooled faeces and expressed as eggs per gram (epg) of faeces. At the termination of the experiment, animals were euthenized

humanely and the intestines removed immediately for the recovery of worms according to the method of Fakae et al. (1994).

Preparation of parasites somatic antigenic extracts and monitoring of antibody response (serum IgG) by ELISA were as described elsewhere (Fakae et al., 1994). Briefly, serum samples were diluted at 1 : 50 while the enzyme conjugate, IgG/peroxidase (goat anti-mouse), was used at 1 : 2000 dilution. Colour reaction was developed with 3,3′,5,5′-tetramethyl benzidine (Sigma). The optical density was read on a Titertek Multiscan (Labsystems) at 450 nm.

2.5. Statistics

Statistical analysis was carried out using Instat (GraphPAD Software, Inc, USA). Non parametric analyses were used according to Fowler and Cohen (1990). Mann-Whitney *U*-test compared pairs of groups for significant differences. Probability (*p*) values less than 0.05 were considered significant.

3. Results

All the mice infected with *H. polygyrus* alone and all the uninfected mice survived until the end of the experiment. Significantly more ($U = 12.0$, $p = 0.0379$) of the immune mice concurrently infected with both parasites survived in the group infected simultaneously than in the similar group challenged with *H. polygyrus* 10 days after the *T. congolense* challenge. Indeed all but one of the immune mice in which *T. congolense* infection preceded *H. polygyrus* challenge died between 13–27 days after infection (DAI) and the last mouse was examined *post-mortem* on Day 28. However, deaths occurred much sooner in the naive mice (Fig. 1).

There was little change in the live weights of either the uninfected mice or those infected with *H. polygyrus* alone. The mean live and eviscerated carcass weights of the five mice that were killed at the beginning of the study were 24.7 and 18.7 g, respectively (i.e. carcass weight was 75.7% of live weight). The difference between the estimated eviscerated carcass weights at the start of the experiment and the actual eviscerated carcass weights at the end of the experiment are shown in Table 2. The concurrently infected mice suffered the most severe weight loss, while most other groups of mice gained some weight as compared to Day 27. The controls gained most weight of all.

The parasitaemias in the mice infected with *T. congolense* ran similar courses (Fig. 2). Unlike the uninfected mice and those infected with *H. polygyrus* alone, all the trypanosome-infected mice became anaemic. The PCV's in immune mice, concurrently challenged and in those with only the *T. congolense* infection dropped by 12.6% and 19.4% at 14 DAI, respectively, remaining at this level or with only slight recovery. The PCV's of both immune and naive mice given *T. congolense* infection 10 days before *H. polygyrus* infection fell consistently until they died, the fall being most rapid in the naive mice (Fig. 3).

There was a clear difference between the faecal worm egg counts of immune and naive mice with counts in all the immune groups being similarly depressed. Immune mice had counts not exceeding 10,000 epg while naïve control reached a maximum of 150,000 epg.

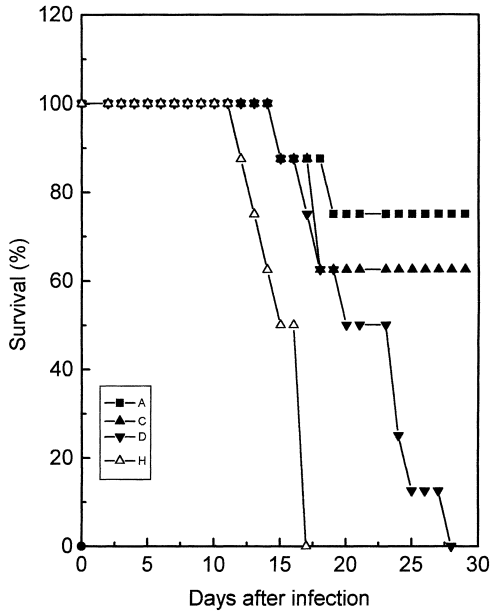

Fig. 1. The mortality of immune (A, C, D) or naive mice (H) challenged with *T. congolense*. Groups A and H were concurrently infected with *H. polygyrus*. Group C was infected with *T. congolense* alone.

Table 2
Eviscerated carcass weights: Mean ± SEM of the estimated eviscerated carcass weights

Group	Mean ± SEM eviscerated carcass weight	Mean ± SEM eviscerated carcass weight (Day 30)
(A) Immune concurrent	20.6 ± 0.4	17.8 ± 0.4[a]
(B) Immune nematode (Day 0)	20.0 ± 0.5	20.6 ± 0.4
(C) Immune Trypanosome (Day 0)	20.1 ± 0.3	20.6 ± 1.0
(E) Immune nematode (Day 10)	20.2 ± 0.3	20.0 ± 0.4
(F) Immune control	20.2 ± 0.3	22.8 ± 0.5[a]
(G) Naive nematode (Day 0)	20.1 ± 0.2	20.9 ± 0.3
(I) Naive control	19.7 ± 0.4	21.9 ± 0.6[a]

[a] Significant difference between starting and terminal weights ($p \leq 0.05$).

All but one of the eight immune mice challenged with *H. polygyrus* on Day 0 were completely protected against the challenge infection. The number completely protected dropped to five when the challenge nematode infection was administered on Day 10 (Table 3). With the exception of one apparent ‘non-responder’ mouse in the immune group (B) given the nematode challenge on Day 0, none of the immune mice had worm burdens in the same range as that of the naive controls. This was even true of the one surviving mouse from the immune group (D) in which the *T. congolense* infection preceded the *H. polygyrus* infection. In immune mice, however, there was no significant difference in the level of protection between concurrently infected mice and those challenged *with H. polygyrus* alone ($p < 0.05$).

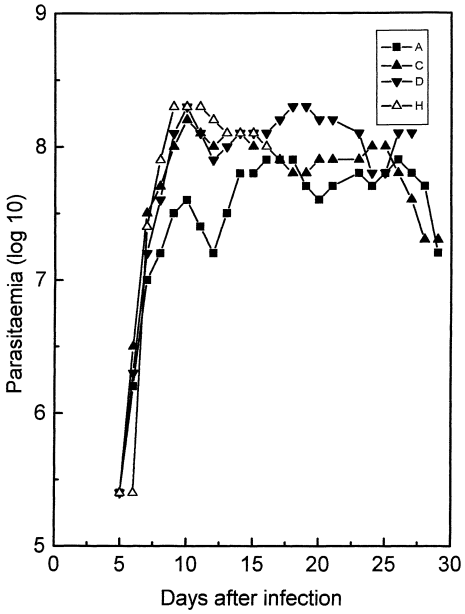

Fig. 2. The mean (SEM ≤ 0.3) *T. congolense* parasitaemia of immune (A, C, D) or naive mice (H) challenged with *T. congolense*. Groups A and H were concurrently with *H. polygyrus*. Group C was infected with *T. congolense* alone.

Table 3
Heligmosomoides polygyrus worm recoveries from mice surviving until the end of the experiment and the level of protection afforded by pre-exposure and drug treatment of larval *H. polygyrus* infection

Group	No. completely protected	Worms recovery (Mean ± SEM)	Protection (%) (Mean ± SEM)
(A) Immune concurrent	0/6[a]	45.2 ± 19	84.3 ± 7
(B) Immune nematode (Day 0)	7/8	25.1 ± 24	91.2 ± 8
(D) Immune split challenge	0/1[b]	134	53.3
(E) Immune nematode (Day 10)	5/8	20.4 ± 12	92.9 ± 4
(G) Naïve nematode(Day 0)	0/8	286.9 ± 17	0

[a] Two of these mice had only one worm each.
[b] This mouse was examined during *post-mortem* one day earlier.

There was a rapid rise in the antibody (total IgG) response to *H. polygyrus* antigens in the sera of immune mice following challenge with *H. polygyrus* alone. By Day 28, the antibody titres in such mice challenged with nematodes on Day 0 were clearly higher ($U = 6$, $p = 0.01$) than in immune mice concurrently infected on that day (Fig. 4). Mice in which *T. congolense* infection preceded *H. polygyrus* infection did not display significant antibody responses.

The antibody response to *T. congolense* antigens by immune mice infected with *T. congolense* alone or concurrently with *H. polygyrus* rose rapidly and progressed throughout the infection (Fig. 5). There was a similar initial rise in the antibody titres in immune mice in which *T. congolense* infection preceded the *H. polygyrus* infection but the titres in the

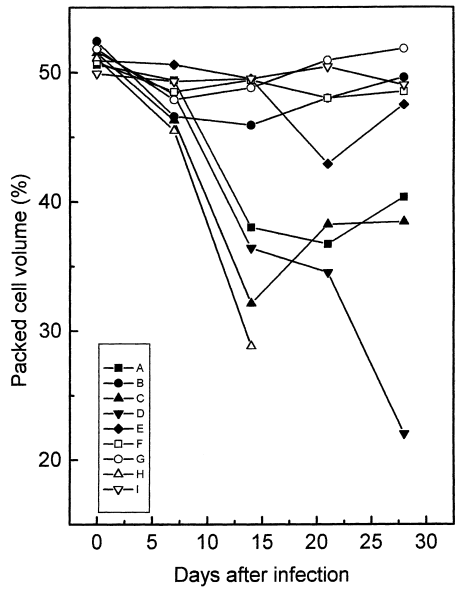

Fig. 3. The mean (SEM ≤ 2.9) PCVs of immune (A, B, C, D, E and F) or naive (G, H and I) mice infected with *T. congolense* alone (C) or *H. polygyrus* alone (B and E) or concurrently infected with both parasites (A, D and H). Groups F and I were uninfected immune and naïve controls, respectively.

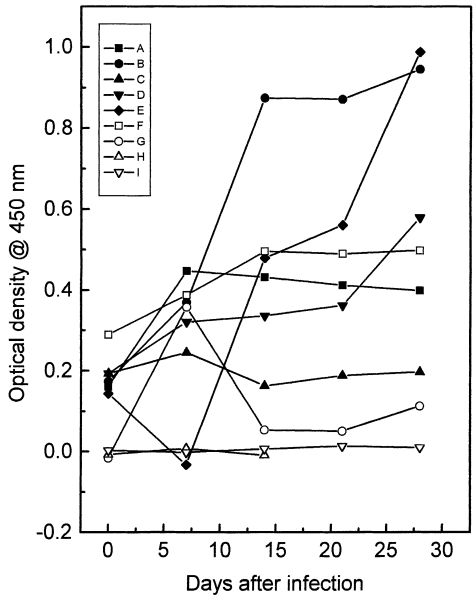

Fig. 4. The mean antibody titres against *H. polygyrus* (SEM ≤ 0.13) of immune (A, B, C, D, E and F) or naive (G, H and I) mice infected with *T. congolense* alone (C) or *H. polygyrus* alone (B and E) or concurrently infected with both parasites (A, D and H). Groups F and I were uninfected immune and naïve controls, respectively.

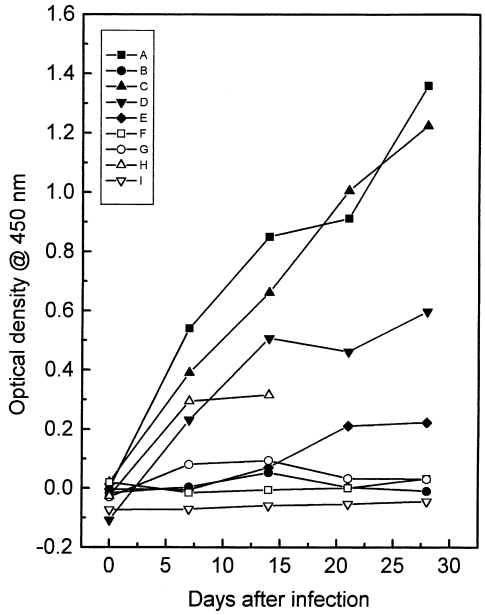

Fig. 5. The mean antibody titres against *T. congolense* (SEM $\leq$ 0.12) of immune (A, B, C, D, E and F) or naive (G, H and I) mice infected with *T. congolense* alone (C) or *H. polygyrus* alone (B and E) or concurrently infected with both parasites (A, D and H). Groups F and I were uninfected immune and naïve controls, respectively.

former group remained almost unaltered from 14 DAI (i.e. 4 days after *H. polygyrus* infection). Naive mice and those infected with *H. polygyrus* alone did not exhibit an antibody response to *T. congolense*.

4. Discussion

These results confirmed earlier findings (Fakae et al., 1994) that concurrent *H. polygyrus* and *T. congolense* infection is detrimental to the TO mice. They further demonstrated that increased mortality and weight losses and interference with acquired immunity against *H. polygyrus* were associated with infections in which the *T. congolense* infection preceded *H. polygyrus* infection. However, the findings suggest that in contrast to mice immunised by termination of adult worms (Fakae et al., 1997), those immunised by abbreviated larval infection may not be severely compromised. Truly, protection acquired by truncated adult *H. polygyrus* infection could be regarded as inferior in that the adult worm themselves have been shown to suppress functional protective immunity to challenge infection (Pleass and Bianco, 1994).

Concurrent infection did not have any apparent effect on the egg outputs of immune mice, this being a reflection of the similar worm burdens in these mice. True comparisons of the worm burdens in the controls and in the groups in which *T. congolense* infection preceded *H. polygyrus* infection are impossible because all but one of the mice with such dual infections died before *post-mortem* worm counts were performed. The only evidence

from the single surviving immune mouse in the immune group given a split reinfection suggests that up to 40% of the expected protection against *H. polygyrus* was lost when *T. congolense* infection preceded the nematode infection.

Studies with other host–parasite systems have shown that some haemoprotozoans are capable of interfering with the development of resistance to a homologous helminth challenge. Both acute malaria (*Plasmodium berghei*) and trypanosome (*Trypanosoma brucei*, TREU 792) infections initiated at the same time as *Trichuris muris* infection suppressed the mechanisms responsible for immune expulsion of the nematode (Phillips et al., 1974). Similar effects due to *T. brucei* were also observed in mice with *Echinostoma revolutum* infection (Christensen et al., 1984). In the present study, except when *T. congolense* preceded the *H. polygyrus* secondary infection, immune mice retained substantial protection under concurrent infection. This suggests that *T. congolense*-induced interference with acquired immunity to *H. polygyrus* infection in mice is influenced, among other things, by the duration of the *T. congolense* infection prior to the challenge *H. polygyrus* infection. It may be of interest to study, in this model, the dynamics of T-cells and the repertoire of cytokines that have been shown to regulate host defense (Finkelman et al., 1997; Grencis, 1997; Else and Finkelman, 1998).

Although *H. polygyrus* has been shown to depress the immune response to heterologous antigens (Chowaniec et al., 1972; Ali and Behnke, 1983), there have been no reports of *H. polygyrus* enhancing the pathogenicity of a blood protozoan. Bell et al. (1984) reported that, although concomitant infection with *Trichinella spiralis* in the mouse increased maximum *Trypanosoma musculi* parasitaemia by two- to four-fold, regardless of the degree of resistance of the murine strain to either *T. musculi* or *T. spiralis*, *H. polygyrus* did not promote a *T. musculi* parasitaemia over the level of a single infection. In the present study, although the levels of *T. congolense* parasitaemia in singly and dually infected mice were identical, there was clear potentiation of the pathogenicity of *T. congolense* as indicated by the clinicopathological parameters including mortality, eviscerated carcass weights and serum antibody response, especially when *H. polygyrus* infection was superimposed on a pre-existing protozoan infection. Kaufmann et al. (1992) also observed that, in N'Dama cattle, *Haemonchus contortus* did not influence *T. congolense* parasitaemia but drastically increased weight losses and mortality in animals in which trypanosomes were present prior to infection with the nematode, this being the most harmful combination. In the present study, the pathological effects of *T. congolense* on the mice may have been exacerbated by the disruptive effects of the *H. polygyrus* larvae, whose development in the intestine produces most of the pathological lesions in heligmosomoidosis (Liu, 1965).

The synergistic effects of *H. polygyrus* on the *T. congolense* infection might have also arisen from immunodepression of the host's response to the protozoan caused by *H. polygyrus*, as demonstrated by the decreased anti-*T. congolense* serum antibody titres in those mice which received *H. polygyrus* 10 days after the *T. congolense* infection. The mechanisms whereby such immunodepression is mediated in this system are not understood. However, it has been shown that *H. polygyrus* secretes immunomodulatory factor(s) (Behnke et al., 1983; Losson et al., 1985; Monroy et al., 1989), which possibly facilitate the parasite's survival in the host. Both in vitro and in vivo studies have also shown that soluble antigens in a homogenate of *H. polygyrus* depress the response to heterologous sheep red blood cells in mice (Pritchard et al., 1984; Crawford et al., 1989).

Although the protective responses against homologous challenge produced in mice by abbreviation of *H. polygyrus* adult infection were completely lost as a result of concurrent infection with *T. congolense* (Fakae et al., 1997) the stronger protection produced in mice by an abbreviated larval infection in the present study was merely reduced. This suggests that animals with a strong immunity against gastrointestinal nematodes may largely overcome the suppressive influence of trypanosomes on a homologous challenge. If the capacity of trypanosome as a general non-specific immunosupressor can be limited in the definitive hosts by a superior acquired immunity against homologous nematode as observed in this study, prospects of control through selective breeding of high responders in trypanosome endemic area may be feasible.

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References

Ali, N.M.H., Behnke, J.M., 1983. *Nematospiroides dubius*: factors affecting the primary response to sheep red blood cell in infected mice. Journal of Helminthology 57, 343–353.

Behnke, J.M., Hannah, J., Pritchard, D.I., 1983. *Nematospiroides dubius* in the mouse: evidence that adult worms depress the expression of homologous immunity. Parasite Immunology 5, 397–408.

Bell, R.G., Adams, L.S., Ogden, R.W., 1984. *Trypanosoma musculi* with *Trichinella spiralis* or *Heligmosomoides polygyrus*: Concomitant infections in the mouse. Experimental Parasitology 58, 8–18.

Chiejina, S.N., 1986. The epizootiology and control of parasitic gastro-enteritis of domesticated ruminants in Nigeria. Helminthological Abstracts 55, 413–429.

Chiejina, S.N., 1987. Some parasitic diseases of intensively managed West African Dwarf sheep and goats in Nsukka, eastern Nigeria. British Veterinary Journal 143, 246–272.

Chowaniec, W., Wescott, R.B., Congdon, L.L., 1972. Interaction of *Nematospiroides dubius* and influenza virus in mice. Experimental Parasitology 32, 33–44.

Christensen, Ñ., Fagbemi, B.O., Nansen, P., 1984. *Trypanosoma brucei*-induced blockade of expulsion of *Echinostoma revolutum* and homologous *E. revolutum* resistance in mice. J. Parasitol. 70, 558–561.

Crawford, C., Behnke, J.M., Pritchard, D.I., 1989. Suppression of heterologous immunity by *Nematospiroides dubius* antigens in vitro. International Journal for Parasitology 19, 29–34.

Else, K.J., Finkelman, F.D., 1998. Intestinal parasites, cytokines, and effector mechanisms. International Journal for Parasitology 28, 1145–1158.

Fabiyi, J.P., 1987. Production losses and control of helminth in ruminants of tropical regions. International Journal for Parasitology 17, 435–442.

Fakae, B.B., 1990. Epidemiology of helminthosis of small ruminants under the traditional husbandry system in eastern Nigeria. Veterinary Research Communications 14, 381–391.

Fakae, B.B., 1993. *Conjoint Heligmosomoides polygyrus*and *Trypanosoma congolense*infection in mice. PhD Thesis, The University of Edinburgh, pp. 1–219

Fakae, B.B., Chiejina, S.N., 1993. The prevalence of concurrent trypanosome and gastrointestinal nematode infections in West African dwarf sheep and goats in Nsukka area of Eastern Nigeria. Veterinary Parasitology 49, 313–318.

Fakae, B.B., Chiejina, S.N., Behnke, J.M., Ezeokonkwo, R.C., Nnadi, P.A., Onyenwe, W.I., Gilbert, F.S., Wakelin, D., 1999. The response of Nigerian West African Dwarf goat to experimental infection with *Haemonchus contortus*. Research in Veterinary Science 66, 147–158.

Fakae, B.B., Harrison, L.J.S., Ross, C.A., Sewell, M.M.H., 1994. *Heligmosomoides polygyrus* and *Trypanosoma congolense* infections in mice: a laboratory model for concurrent nematode and trypanosome infections. Parasitology 108, 61–68.

Fakae, B.B., Harrison, L.J.S., Ross, C.A., Sewell, M.M.H., 1997. Expression of acquired immunity to Heligmosomoides polygyrus infection in mice concurrently infected with *Trypanosoma congolense*. International Journal for Parasitology 27, 1107–1114.

Finkelman, F.D., Shea-Donohue, T., Goldhill, J., Sullivan, C.A., Morris, S.C., Madden, K.B., Gause, W.C., Urban Jr., J.F., 1997. Cytokine regulation of host defence against parasitic gastrointestinal nematodes: lessons from studies with rodent models. Annual Review of Immunology 15, 505–533.

Fowler, J., Cohen, L., 1990. Practical Statistics for Field Biology. Milton Keynes, Open University Press.

Gray, G.D., 1997. The use of genetically resistant sheep to control nematode parasitism. Veterinary Parasitology 72, 345–366.

Grencis, R.K., 1997. Th2-mediated host protective immunity to intestinal nematode infections. Philosophical Transactions of the Royal Society of London Series B–Biological Sciences 352, 1377–1384.

Herbert, W.J., Lumsden, W.H.R., 1976. *Trypanosoma brucei*: a rapid 'matching' method for estimating the host's parasitaemia. Experimental Parasitology 40, 427–431.

Kaufmann, J., Dwinger, R.H., Hallebeek, A., van Dijk, B., Pfister, K., 1992. The interaction of *Trypanosoma congolense* and *Haemonchus contortus* infections in trypanotolerant N'Dama cattle. Veterinary Parasitology 43, 157–170.

Klei, T.R., 1997. Immunological control of gastrointestinal nematode infections. Veterinary Parasitology 72, 505–523.

Liu, S.K., 1965. Pathology of *Nematospiroides dubius* I. Primary infections in C3 and Webster mice. Experimental Parasitology 17, 123–135.

Losson, B., Lloyd, S., Soulsby, E.J.L., 1985. *Nematospiroides dubius*. Modulation of the host immunological response. Zeitschrift für Parasitenkunde 71, 383–393.

Monroy, F.G., Dobson, C., Adams, J.H., 1989. Low molecular weight immunosuppressors secreted by adult *Nematospiroides dubius*. International Journal for Parasitology 19., 125–127.

Murray, M., Morrison, W.I., Emery, D.L., Akol, G.W.O., Masake, R.A., Moloo, S.K. 1980. Pathogenesis of trypanosome infection in cattle. In: Isotope and Radiation Research on Animal Diseases and Their Vectors, International Atomic Energy Agency (IAEA-SM-240/19), pp. 15–32.

Phillips, R.S., Selby, G.R., Wakelin, D., 1974. The effect of *Plasmodium berghei* and *Trypanosoma brucei* infections on the immune expulsion of the nematode *Trichuris muris* from mice. International Journal for Parasitology 4, 409–415.

Pleass, R.J., Bianco, A.E., 1994. The role of adult worms in suppressing functional protective immunity to *Heligmosomoides polygyrus bakeri* challenge infections. Parasite Immunology 16., 619–628.

Pritchard, D.I., Ali, N.M.H., Behnke, J.M., 1984. Analysis of the mechanism of immunosuppression following heterologous antigenic stimulation during concurrent infection with *Nematospiroides dubius*. Immunology 51, 633–642.

Wahid, F.N., Behnke, J.M., 1992. Stimuli for acquired resistance to *Heligmosomoides polygyrus* from intestinal tissue resident L3 and L4 larvae. International Journal for Parasitology 22, 699–710.

Wakelin, D., 1984. Immunity to parasites. In: How Animals Control Parasite Infections, Arnold, 160 pp.