Interaction between *Trypanosoma brucei* and *Haemonchus contortus* infection in West African Dwarf Goats

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**ABSTRACT**

In order to investigate the immunomodulatory influence of concurrent *T. brucei* and *H. contortus* infection in West African Dwarf (WAD) goats, 28 infected and 7 uninfected (control) of 8-9 months old male WAD goats were studied. The infected goats were separated into resistant (Class 1) and susceptible (Class 2) Faecal Eggs Count (FEC) phenotypes. They were infected with 3000 infective larvae (L₃) of *H. contortus* and 5 x 10⁶ *T. brucei*. Another group of these phenotypes received only the *H. contortus* challenge. The responses were measured by parasitological response through FEC and Worm Burden (WB), and by host immune response through IgG levels. Class 2 animals with or without *T. brucei* infection exhibited non-significant (P > 0.05) higher FEC compared to those of Class 1. Worm burdens were not significantly different between FEC classes but were different (P=0.038) in relation to *T. brucei* infection. There was an overall increase in worm burdens of *T. brucei* infected goats, more pronounced in Class 2 than in Class 1. There was a significant upward drift of antibody levels with time across all groups without significant interactions. However, there was a significant effect of FEC Class (P=0.017) and trypanosome infection (P=0.041) with no significant interaction. Animals in Class 1 had generally higher antibody levels than those in Class 2, irrespective of *T. brucei* infection. There was an overall increase in worm burdens of *T. brucei* infected goats, more pronounced in Class 2 than in Class 1. There was a significant upward drift of antibody levels with time across all groups without significant interactions. However, there was a significant effect of FEC Class (P=0.017) and trypanosome infection (P=0.041) with no significant interaction. Animals in Class 1 had generally higher antibody levels than those in Class 2, irrespective of *T. brucei* infection. Infection status had a highly significant (P=0.0001) positive correlation with IgG. *H. contortus* only infected goats had the highest antibody levels and trypanosome infection reduced this response, irrespective of FEC phenotype. There was a highly significant (P=0.001) positive correlation (r = 0.719) between FEC and total worm counts. This work showed that the two response phenotypes identified in earlier immunizing infections were still clearly recognizable following homologous challenge and dual infections. This suggests that under field conditions where repeated *H. contortus* challenge occurs and trypanosomosis is endemic, the phenotypes could remain unchanged.

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**Keywords:** Haemoparasite, Gastrointestinal parasite, concurrent infection, immune response, domestic animals.

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DOI: http://dx.doi.org/10.4314/ijbcs.v10i4.11

2643-IJBCS
INTRODUCTION
Gastrointestinal parasites is the major health problem of grazing animals in tropical, as well as in temperate regions (Azando et al., 2011; Cardoso et al., 2013; Okombe et al., 2013), including West African Dwarf (WAD) goats. *H. contortus* is the most pathogenic, being a large haematophagous blood-sucking parasite (Olounladé et al., 2011) that can induce severe anaemia and death. Worm problems are greatest in areas with an annual rainfall of 350 mm or greater as in the sub humid zone of Nigeria and Adamawa region in Cameroon. *H. contortus* is susceptible to cold and desiccation and predominates in the Northwest Region of Cameroon (Ndamukong and Sewell, 1992) and the second most numerous gastrointestinal helminthes of ruminants in Adamawa region (Ebene et al., 2011). The problem is exacerbated by a widespread and growing problem of anthelmintic resistance in worm populations (Varady et al., 2011; Bartram et al., 2012; Rialch et al., 2013; Yèïnou et al., 2015). This makes ongoing reliance on chemical control of GIN a risky and possibly non-sustainable approach. Selection for genetic resistance in the host is an alternative control measure, which could be used in conjunction with chemical, and other control measures to place worm control on a more sustainable footing into the future. Polyparasitism is the most common form of animal parasitosis in tropical zones (Chiejina, 2001). Of particular interest are concurrent infections involving parasites from unrelated groups such as helminthes and protozoan (Christensen et al., 1987). Although helminthosis and trypanosomosis are both endemic in tropical Africa, most studies and surveys have examined these diseases as separate entities, ignoring the fact that they frequently occur as concurrent infections (Fakae and Chiejina, 1993). Concurrent gastrointestinal nematode-trypanosome infections have practical implications for livestock production in endemic regions, especially where diagnostic and control methods are poorly developed. These include suppression of host immunological responses to the nematode parasite (Kaufman et al., 1992) and suppression of host responses to vaccines used for the control of some well-known livestock diseases (Ilemobade et al., 1982; Rurangirwa et al., 1983; Scott et al., 1997). Most of our current knowledge on the phenomenon of trypanosome immunosuppression in concomitant nematode infections has been derived largely from studies in laboratory animal models (Fakae et al., 1997), such as *Trichinella spiralis* (Chiejina and Wakelin, 1994; Onah and Wakelin, 2000), *Nippostrongylus brasiliensis* (Wedrychowicz et al., 1984), but also in some studies in farm animals (Kauffman et al., 1992; Gossens et al., 1997; Faye et al., 2002). However, the full extent of the impact of concurrent helminth–protozoal infections on livestock production remains to be fully understood. These include the mechanisms by which trypanosomes suppress anti-nematode resistance, and the degree to which the level of suppression seen in concurrent infections is influenced by genetically determined variation in the hosts and the capacity to respond to each of the infections involved.

Relatively recent studies have shown that the WAD goat of the Nigerian sub humid zone has the capacity to express strong resistance and resilience to its most important gastrointestinal nematode parasite, *H. contortus* (Fakae et al., 2003; Behnke et al., 2006). This attribute, which has been termed haemonchotolerance exhibits marked individual variability in which two distinct responder phenotypes, namely strong and weak have been identified (Behnke et al., 2006), using phenotypic markers such as FEC and Packed Cells Volume (PCV). Goats in the strong responder class are able to control moderate to heavy infections without significant pathology and pass very few eggs in their faeces. This class forms 75-80% of the goat population. The weak responders, which are relatively more susceptible, are likely to show clinical symptoms of haemonchosis even in moderate infections (Behnke et al., 2006).

Thus, it is possible to segregate WAD goats into responder phenotypes using FEC. This simple measure of infection was used to segregate the two classes of goats used in this study, following prolonged and graded escalating infections. The objectives of this work were to find out: (1) whether the clear-
cut segregation between the two phenotypes would still be maintained in the face of homologous H. contortus challenge; (2) what influence concomitant H. contortus–Trypanosoma brucei would have on the expression of the two response phenotypes.

MATERIALS AND METHODS
Experimental protocol
Twenty-eight infected and seven uninfected (control) 8–9 months old, male WAD goats were studied. The infected (H. contortus immunized) goats were separated into fourteen resistant (Class 1) and fourteen susceptible (Class 2) FEC phenotypes. Seven goats from the resistant (Group 1A) and seven from susceptible (Group 2A) FEC phenotypes received concurrent infection with 3000 L3 of H. contortus and 5 x 106 T. brucei. Similar numbers of these phenotypes (Group 1B and Group 2B) received only the H. contortus challenge. The control group remained T. brucei naïve. This work utilized two measures of parasitological response (FEC and WB) and IgG as a measure of host immune responsiveness.

Management of animal
All the 35 animals used in this study were males. They were housed in groups of seven in concrete-floored pens, which were debated with dry sawdust. They were fed three times daily on specially formulated concentrate and fresh grass cut from known helminth-free paddocks. Water was provided ad libitum. Other management routines designed to ensure a high standard environmental hygiene in the animal house and prevent unwanted helminth infections were introduced during the period of the experiment.

Parasites

Haemonchus contortus

Infective larvae (L3) of H. contortus were harvested from faecal cultures prepared from donor goats infected with a pure strain of the nematode as described by Fakae et al. (1999) and Musongong et al. (2003). The L3 were stored at 4 °C and used within three weeks of harvest from cultures.

Trypanosoma brucei

T. brucei freshly isolated from a clinical case of canine trypanosomosis in Nsukka was maintained in trypanosome naïve outbred strain of albino mice. It was subsequently passaged every 2 weeks in other naïve mice. Three passages mice showing parasitaemia of log108.0 were deeply anaesthetized with Diethyl ether. One millilitre of heart blood was quickly taken from each mouse and transferred into a bottle containing 500 µl ice-cold Phosphate Buffered Saline (PBS), pH 7.4, which contained 2 µl heparin. The parasitaemia of the collected blood was assessed by the matching method of Herbert and Lumsden (1976) and adjusted as necessary to obtain a working dose of 5 x 106 organisms in 0.3 ml of blood. Each of the 14 T. brucei infected goats (Group 1A and Group 2A) received this dose by subcutaneous inoculation on day 0. Every stage of preparation of the working dilution of blood in PBS was carried out on ice.

Serum collection and IgG antibody determination

Following infections, blood samples were collected in order to assess pathological consequences and/or the host immune response after the artificial challenge. All animals were bled on days 0, 5, 10, 17, 25 and 32. Jugular vein blood samples (1 ml) were collected in EDTA coated tubes and plasma was separated by centrifugation and stored at -20 °C until required for enzyme linked immunosorbent assay to determine the levels of specific IgG to H. contortus antigens in each sample as described by Fakae et al. (2003). Optical densities (OD) were read using Dynatech MR 700 automatic ELISA reader. The OD of each serum sample was expressed as a percentage of the OD value observed in a positive pool.

Statistical analysis

Statistical analyses were carried out using SPSS for windows, version 15. FEC and worm count data did not conform to normal distribution, appropriate transformation were adopted namely, log10(X + 25) for FEC and log10(X + 10) for worm counts (Fakae, et al., 2003) prior to analysis. FEC and IgG which
were recorded on more than one occasion were analyzed by repeated measures in general linear model. Results were expressed as mean ± sem. Correlations between variables were analyzed by Spearman’s Rank Order Test.

RESULTS

Faecal egg count

As can be seen in Figure 1, there was a tendency for goats in Class 2, with or without *T. brucei* infection, to have higher FEC than their counterparts in FEC Class 1. However, this difference was not significant (P > 0.05). Although there was also a tendency for FEC to increase with time, there was no significant effect of time nor was there a significant interaction of time with FEC class or *T. brucei* infection.

Worm burdens

The analysis showed that worm burdens were not significantly different between FEC classes but were significantly different in relation to *T. brucei* infection (P = 0.038). There was an overall increase in worm burdens of *T. brucei* infected goats but this effect was more pronounced in FEC Class 2 than in Class 1 phenotype (Figure 2).

Serum IgG response

There was a significant upward drift of antibody levels with time across all groups (Figure 3), with no significant interactions. However, analysis on between subjects effects, with FEC class and trypanosome infection as factors, revealed a significant effect of FEC class (P = 0.017) and trypanosome infection (P = 0.041), with no significant interactions. Animals in FEC Class 1 had generally higher antibody levels than those in FEC Class 2, irrespective of trypanosome infection (Figure 3). One-way ANOVA, with infection status as the factor, showed clearly that infection status had a very highly significant effect (P = 0.0001). It can be seen from Figure 3 that *Haemonchus* only infected goats had the highest antibody levels and that trypanosome infection reduced this response, irrespective of FEC phenotype.

Correlation between different measures of infection

There was a highly significant (P=0.001) and positive correlation (r = +0.719) between FEC and total worm counts at autopsy on day 38 (Figure 4).

![Figure 1: Effect of concurrent trypanosome- *Haemonchus* infection on Faecal. Eggs Count strong (Class 1) and weak (Class 2) responders in West African Dwarf goats with or no *Trypanosoma brucei* infection.](image-url)
Figure 2: Mean worm counts of strong (Class 1) and weak (Class 2) African Dwarf goat Faecal Eggs Count responders with or no Trypanosoma brucei infection.

Figure 3: Effect of concurrent trypanosome-Haemonchus infection on IgG level in strong (Class 1) and weak (Class 2) West African Dwarf goats Faecal Eggs Count responders with or no Trypanosoma brucei infection.
DISCUSSION

These results show that there was a tendency for goats in the FEC Class 2 to have higher FEC than their counterparts in FEC Class 1 although no significant difference was found between the two classes, whether infected or not with *T. brucei*, and this difference did not change with time. This supports the hypothesis that despite the *H. contortus* homologous challenge and the concurrent *H. contortus*-*T. brucei* infections, the parasitological responses which formed the basis for segregation of these goats into the two FEC classes would be largely maintained. The lack of a significant difference observed in the FEC class phenotypes could possibly have been a result of the modulatory effect of host-acquired immunity after abbreviation of *H. contortus* infection with fenbendazole. Fakae et al. (2003) observed that acquired immunity altered the FEC responses of many but not all goats in FEC Class 2 before anthelmintic treatment and challenge infection. Nevertheless, the initial phenotypes remained the same.

The wide Eggs Per Gram (EPG) variations in the overall responses, following *H. contortus* infection in these goats may be associated with several factors such as variations in the innate ability of individual WAD goats to develop resistance to the parasite (Stear and Wakelin, 1998; Fakae et al., 2003). The genetic basis of variability in responsiveness to infection has also been found in mice infected with *Trichuris muris* (Else et al., 1990) and *Heligmosomoides polygyrus* (Wahid and Benhke, 1993).

It is noteworthy that *T. brucei*, which is known to suppress immune response to GI nematodes (Chiejina et al., 2003), resulting in increased faecal egg output, did not have this effect. One possible explanation is that the WAD goat is a well-known trypanotolerant breed (Behnke et al., 2006), thus responding in a similar manner, regardless of the FEC phenotype.

Worm burdens were not significantly different between FEC classes but were different in relation to *T. brucei* infection as shown by the overall increase in worm burden of *T. brucei* infected goats, with these effects being more pronounced in FEC Class 2 than in the FEC Class 1. Immunosuppression caused by *T. brucei* infection was probably responsible for the increase in the

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**Figure 4**: Correlation between worm burden and Faecal Eggs Count ($r = +0.791; P = 0.001; n = 35$).
establishment of worms in the *T. brucei*-infected groups. The fact that the two phenotypes did not respond similarly with respect to establishment of worms in *T. brucei*-infected groups supports the hypothesis that the two phenotypes would still remain separate despite *H. contortus* homologous challenge and concurrent *T. brucei* infection. Thus, WAD goats, regardless of their FEC response phenotype, are susceptible to *T. brucei*-induced immunomodulation, but this effect is more pronounced in the weak responders.

 Concurrent *T. brucei* infection has been shown to enhance worm establishment, survival, development and worm fecundity in other host parasite systems, notably in mice concurrently infected with *H. Polygyrus* and *T. congolense* (Fakae et al., 1997), in rats infected with concurrent *T. brucei* and *Nippostrongylus brasiliense* (Wedrychowicz, et al., 1984) and in mice concurrently infected with *T. brucei* and *Trichinella spiralis* (Onah and Wakelin, 2000) and in goats (Sharma et al., 2000). This effect is believed to be immunologically mediated through Th2-dependent responses involving IL4 and IL5 and hence mast cells and eosinophils respectively (Finkelman et al., 1997). Although the precise mechanisms involved in immune suppression is poorly understood, there is strong evidence of Th-1/Th-2 dichotomy in immune responses to GI nematode infections in sheep (Gill et al., 2000; Shakya et al., 2009). They have shown that rejection of *H. contortus* by immune sheep is also largely Th-2-dependent. There is therefore, reason to believe that the mechanisms responsible for trypanosome-induced immunosuppression in farm livestock in trypanosome-endemic areas are broadly similar to those that have so far been demonstrated in laboratory model systems. However, it is not clear why it should be more pronounced in weak responder than in strongly haemonchotolerant phenotypes, nor why female worms should be more affected. It is presumed that the greater nutritional demand of gravid female worms could lead to greater ingestion of parasite specific antibodies and other harmful substances in the gastrointestinal tract (Musongong, 1998). *T. brucei* could possibly counteract this process through its significant reduction in the quantity and availability of such antibodies. There is evidence in the present study that concurrent *T. brucei* infection significantly down regulates *H. contortus* specific IgG antibodies. One practical implication of the preferential effect on responder phenotypes is that in the WAD goat population, it is likely that *T. brucei*-induced immunomodulation affects primarily the weak responder phenotypes. It is these that are likely to contribute significantly to pasture contamination with worm eggs. They are also the individuals that are likely to show the additive pathological effects of haemonchosis and trypanosomosis.

**Conclusion**

This work showed that the two response phenotypes identified in earlier immunizing infections were still clearly recognizable following homologous challenge and dual infections. This suggests that under field conditions where repeated *H. contortus* challenge occurs and trypanosomosis is endemic, the phenotypes could remain unchanged. This also suggests that the variability in resistance and resilience to *H. contortus* is an intrinsic characteristic feature of *H. contortus* WAD goat host-parasite relationship. Data also suggest that although endemic trypanosomosis does not abrogate the distinctive response phenotypes, it modulates the differences between the two and significantly enhances the worm burdens of the weak responders. The overall significant depression of *H. contortus*-specific IgG level is evidence of the well-known immunosuppressive influence of *T. brucei* on gastrointestinal nematode infections.

**COMPETING INTERESTS**

The authors declare that they have no competing interests.
AUTHORS’ CONTRIBUTIONS

NJE, SNC, JPM and IOO designed the work plan; NJE and JPM implemented field work and collected samples; NJE, SNC, JPM and IOO interpreted the results; NJE, JPM and MYM compiled, tabulated, analyzed the data and prepared the manuscript. All the authors read and approved the final manuscript.

REFERENCES


Ebene NJ, Onah DN, Nwosu CO, Messine O. 2011. Species diversity of gastrointestinal parasites in the High Guinea Savannah agroecological zone in Cameroon. First Days of the School of Veterinary Medicine and Sciences, 16 to 18th of March. School of Veterinary Medicine and Sciences, University of Ngaoundere, Cameroon.


Fakae BB, Chiejina SN, Behnke JM, Ezeokonkwo RC, Nnadi PA, Onyenwe WI, Gilbert FS, Wakelin D. 1999. The response of Nigeria West African...
Dwarf goats to experimental infections with *Haemonchus contortus*. Research in. Veterinary Science, **66**:147-158.

Fakae BB, Chiejina SN, Behnke JM, Nnadi PA, Musongong GA, Wakelin D. 2002. Expression of acquired immunity to a local isolate of *Haemonchus contortus* by the Nigerian West African Dwarf goat. Veterinary Parasitology, **107**:1-14.


