Studies on the Leucocytic Response to Experimental Infection with *Trypanosoma brucei* and *Haemonchus contortus* in Red fronted gazelles (*Gazella rufifrons*)

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ABSTRACT

The leucocytic response to a concurrent *Trypanosoma brucei* and *Haemonchus contortus* infection following parasitaemia and appearance of ova in faeces in red fronted gazelles (*Gazella rufifrons*) was investigated. A marked decline ($P < 0.05$) in total white blood cell (WBC) and lymphocyte counts was observed among the concurrently infected red fronted gazelles compared to those with single infections. Also, neutrophil numbers declined significantly ($P < 0.05$) in red fronted gazelles infected either singly with *Trypanosoma brucei* or concurrently with both parasites while those infected singly with *Haemonchus contortus* experienced a significant ($P <0.05$) rise in neutrophil counts which became evident from day 30 post infection. Monocytosis was also observed in those infected singly with *Trypanosoma brucei* and concurrently with both parasites and eosinophilia ($P <0.05$) in the gazelles with dual infection or those singly infected with *Haemonchus contortus*. These suggests therefore, that the leucocytic response observed which was more marked following concurrent infection with both parasites in the gazelles, tended more towards immunosuppression.

Key words: Leucocytic response; experimental infection; *Trypanosoma brucei*; *Haemonchus contortus* ; red fronted gazelles

INTRODUCTION

Livestock are often exposed to concurrent infections with several parasite species and the presence of one parasite may cause either a synergistic or an antagonistic effect in the host (Griffith *et al*., 1981a; Nwosu *et al*., 2006). Despite extensive literature search, there is no indication of previous information in wild animals.

Studies on synergistic effects of concurrent infections by Urquhart *et al*., (1973) have shown that a primary *Trypanosoma brucei* infection enhanced the pathogenicity and prevented the mounting of secondary immune responses with a delay in the normal expulsion of a challenge infection of rats with the nematode *Nippostrongylus brazilliansis*. Several authors (Griffith *et al*., 1981a; Griffith *et al*., 1981b; Nwosu *et al*., 2001; Nwosu *et al*., 2006) demonstrated that concurrent *Trypanosoma congolense* infection rendered goats normally resistant to *Haemonchus contortus* more susceptible to the nematode and resulted in more severe infections.

The red fronted gazelle is almost domesticated in the arid zone of North-eastern Nigeria, where it is kept together with sheep and goats in the same holding pens as an alternative source of protein and in some cases for exhibition purposes. Recently outbreaks of trypanosomosis due to *Trypanosoma brucei* infection in red fronted gazelles (*Gazella rufifrons*) primarily associated with stress in Maiduguri and Abuja zoos were observed and reported (Mbaya, 2007). All the gazelles examined either at the Maiduguri Zoological Garden or in the wild harboured concurrent *Trypanosoma brucei* and *Trichostrongylid* nematodes (Mbaya and Aliyu, 2007). Feacal cultures revealed that *H. contortus* was the predominant nematode in the infection. This study was further undertaken for the first time to evaluate the leucocytic response to a concurrent *T. brucei* and *H. contortus* infection in the semi domesticated wild ungulate since the two parasites often occur together in the field.

MATERIALS AND METHODS

Experimental animals

Following authorization by the Ministry of Environment Borno State, Nigeria, twenty apparently healthy red fronted gazelles (*Gazella rufifrons*) of both sexes aged between 2 to 3 years and weighing between 20 to 25 kg were obtained at Sanda Kyarimi Park Maiduguri using standard capture techniques. During the acclimatization period, the gazelles were routinely treated with Oxytetracycline (Terramycine long acting®), diminazene aceturate (Berenil®) and morantel (Banminth®) against blood rickettsial organisms, trypanosomes and helminthes respectively. They were housed in concrete floor and fly- proof pens throughout the experiment and fed on wheat bran supplemented with bean husks, guinea corn, chopped sweet potatoes and cucumber while water was provided *ad libitum*

Source of trypanosomes

Trypanosoma brucei (Mkar/84/Nitr/6) used for the study, was obtained from the Nigeria Institute for Trypanosomosis Research (NITR) in Kaduna, Nigeria. It was first isolated in 1984 from a fatal outbreak of porcine trypanosomosis in Mkar in Benue State, Nigeria (Agu and Bajeh, 1986). It was identified based on morphology and negative Blood Inhibition and Infectivity Test (BIIT) then stabilized by four passages in rats before storage in liquid nitrogen. The stabilates were passaged twice in rats and then transferred into donor red Sokoto goats and blood from the infected goats was diluted with phosphate buffered saline solution (pH 7.4). Each gazelle was inoculated via the jugular vein with 0.5 ml of blood from the donors containing $1.5x10^6$ trypanosomes.

Source of Haemonchus contortus

Gravid adult *Haemonchus contortus* females were collected from the abomasums of goats after slaughter and evisceration at the Maiduguri Municipal abattoir washed in several changes of physiological saline and then lightly macerated with a pestle and mortar. The material was then centrifuged at 2,500 rpm for 10 minutes to obtain sediment which was reconstituted into a paste using sterile cow faeces before culture for 10 days at (35^0C) to provide pure culture of *Haemonchus contortus* using the modified Baerman technique (Anon, 1977). The larvae recovered were used to orally infect 2 worms – free red fronted gazelles (*Gazella rufifrons*) at the dose rate of 5,000 and 10,000 infective larvae respectively. The gazelle given the higher dose died of the disease 15 days later whereas the other survived over 40 days. Eggs from the faeces of the surviving gazelle were then routinely cultured and the infective larvae recovered were used immediately.

Experimental design

For the purpose of the experimental infection, the gazelles were divided into four groups (A, B, C, D) of five. Group A was infected with 1.5×10^6 *T. brucei,* Group B was infected orally with 5,000 infective larvae of *H. contortus* using stomach tube. Group C was concurrently infected orally with the same dose of *H. contortus* and *T. brucei* via the jugular vein while those in Group D served as uninfected controls.

Monitoring of parasitaemia

Parasitaemia due to *T. brucei* was first detected by the wet mount and buffy coat microscopy method (Murray *et al*., 1983) while counts were estimated by the rapid matching technique of Herbert and Lumsden (1976).

Faecal and haematological examination

Faeces from experimental gazelles were examined every other day as at day 10 post infection for *Haemonchus* ova by the floatation technique using saturated sodium chloride solution as the floating medium (Anon, 1977). Total white blood cells (WBC) and differential leucocytic count (DLC) were determined as described by Schalm *et al*., (1995).

Statistical analysis

Data obtained from the study were summarized as means ± standard deviation and the differences between the means determined at the 95% confidence limit using the analysis of variance (Graph pad Instat. 2000).

RESULTS

Following the establishment of parasitaemia by day 4 post infection in the concurrently infected gazelles and day 8 post infection in those with single infections of *T. brucei* (Figure 1) or the appearance of eggs by day 12 post infection in the faeces of gazelles concurrently infected or by day 16 post infection in those with single *H. contortus* infection (Figure 2), leucocytic changes were observed. WBC counts in groups infected singly with *T. brucei*, *H. contortus* and concurrently with both parasites declined significantly ($P < 0.05$) from pre infection mean values of 14.6 ± 1.91, 14.2 ± 1.88 and 14.4 ± 1.90 respectively and continued unabated to values of 7.2 ± 1.34, 9.7 ± 1.56 and 6.0 ± 1.22 for groups treated singly with *T. brucei*, *H. contortus* or concurrently with both parasites respectively by the end of the experiment. The uninfected group however, maintained fairly constant values throughout the study (Figure 3).

Following infection, lymphocytes populations became reduced ($P < 0.05$) in all infected groups (Figure 4). The decline was progressive and unabated with slight fluctuations in all the infected groups until the end of the experiment. The uninfected control, however, maintained fairly constant lymphocytes values throughout the study period.

Neutrophil numbers however, remained normal in gazelles infected singly with *T. brucei* until day 30 when a sharp drop ($P < 0.05$) was noted. The numbers subsequently maintained a gradual but continuous decline to the end of the study when the lowest values were attained. In contrast, the neutrophil counts fluctuated around the pre-infection values until day 30 post infection when a sharp rise was noted in gazelles infected singly with *H. contortus*. The rise continued unabated until the end of the study when the highest value was recorded. The neutrophil numbers in gazelles with the dual infection of both parasites maintained a steady but significant ($P < 0.05$) decline from the onset of infection to the end of the study when the lowest values were attained in this group. On the other hand, neutrophil numbers remained within the pre-infection levels without any significant changes in the uninfected control group throughout the study period (Figure 5).

In contrast, there was consistent monocytosis ($P < 0.05$) without abatement in groups infected either singly with *T. brucei* and those concurrently infected respectively following infection. Peak values of $28.8 \pm 2.68\%$ and $48.0 \pm 2.68\%$ were attained for the respective groups by the end of the experiment. On the other hand, no significant changes ($P > 0.05$) were however, recorded throughout the study period in gazelles infected singly with *H. contortus* or their uninfected control (Figure 6).

Similarly, after infection, gazelles infected singly with *H. contortus* and those concurrently infected with both parasites, experienced a significant ($P < 0.05$) increase in mean eosinophil counts. The elevation in eosinophil values became evident from days 4 and 18 post infection for gazelles infected concurrently with both parasites and singly with *H. contortus* respectively. This continued unabated till the end of experiment with peak values of 11.4 ± 1.69 and 8.6 ± 1.47 being attained respectively for gazelles infected singly with *H. contortus* and those concurrently infected with both parasites. On the other hand, gazelles infected singly with *T. brucei* remained within their pre-infection levels but showed a slight decline from day 34 till the end of the study. The mean eosinophil values of the uninfected control however, remained fairly constant throughout the period of the study (Figure 7).

DISCUSSION AND CONCLUSION

In this study, total leucocyte numbers did not follow any definite pattern in the gazelles infected singly with *H. contortus* as counts were normal during early periods of the disease but became slightly depressed between days 16 and 26 after which the infection was followed by a slight leucopenia, which was maintained to the end of the study. However, at no period during the disease was the change significant ($P > 0.05$). A similar pattern has been reported in goats infected concurrently with helminthosis and trypanosomosis (Rahman and Collins, 1990; Nwosu *et al*., 2006). Meanwhile, those with dual infection experienced a significant decline ($P < 0.05$) in leucocyte numbers. It is therefore probable that *T. brucei* contributed significantly in the depression of the total leucocytic counts. Although trypanosomosis has been associated with leucocytosis, leucopenia has been more commonly reported (Anosa, 1988). Similarly, corstisolemia due to stress of captivity have been reported to cause leucopenia hence the establishment of *T. brucei* infections in red fronted gazelles (Mbaya, 2007).

The lymphopenia observed in all infected groups which was more marked among those with dual infections are probably due to the increased demand on the system for lymphocytes in both immune and inflammatory responses. On the other hand, neutrophil numbers were increased after day 16 post infection which remained elevated until the end of the study in those in those infected singly with *H. contortus* which agrees with the finding in goats (Nwosu *et al*., 2006).

However, there was a significant (P < 0.05) reduction in lymphocyte and neutrophil numbers in those with the dual infection. The reduction of the neutrophil counts at this point was not clear however, spleenic sequestration of leucocytes as observed in trypanosomosis may have prevented neutrophil numbers from raising (Anosa, 1983). Similarly, consistent monocytosis was encountered but more severely among gazelles with dual infection than those with a single *T. brucei* infection but not in those with a single *H. contortus* infection. Monocytosis is a consistent finding in trypanosomosis and has been reported in *T. brucei* infection of dogs (Nwosu and Ikeme, 1992) and in red fronted gazelles (Mbaya, 2007).

The monocytosis due to trypanosomosis has also been associated with a proliferation of tissue macrophages likely due to the increased demand on the system to remove dead RBC, tissue cells, trypanosomes, antigen – antibody complexes and to participate in immune responses. Since macrophages are formed from blood monocytes, this increased need for macrophages may have been responsible for the consistent monocytosis recorded in this present study. Resistances to most nematode infections, including haemonchosis, have been reported to be mediated through Ige response. Since Ige antibodies are elaborated by eosinophils, most nematode infections have been associated with eosinophilia (Cheijina, 1987).

Similarly, during the study, consequent eosinophilia was recorded in gazelles infected singly with *Haemonchus contortus* or concurrently with both parasites. This observation agrees with those in goats (Nwosu *et al*., (2006). From the foregoing, it is evident that severe leucopenia leading to immuno suppression occurred in the red fronted gazelle thereby compromising their trypanotolerance when exposed to the concurrent infection. It is therefore suggested that screening and treatment of both parasites be carried out when red fronted gazelles are subjected to captivity.

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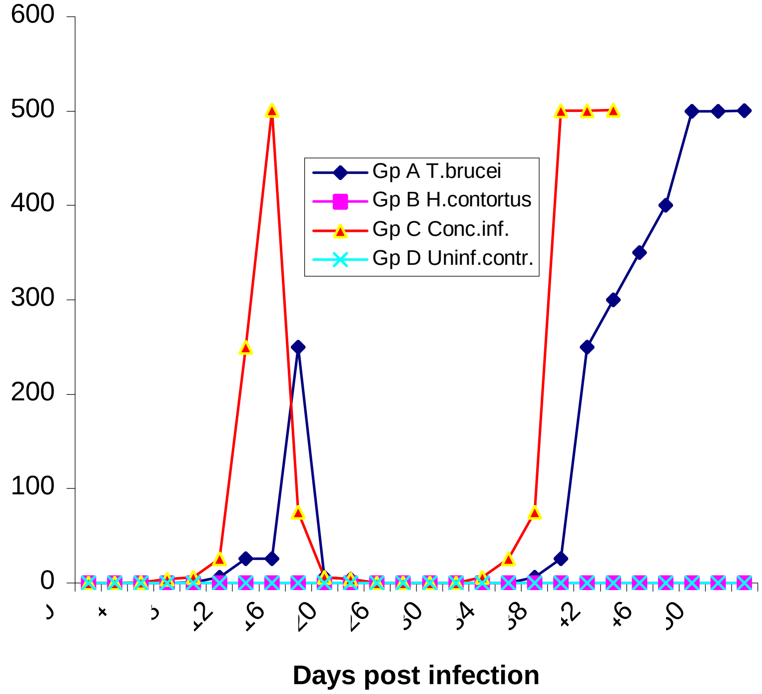

Fig. 1. Mean parasite counts (x 10^6/µl) of red fronted gazelles infected singly with *T. brucei*, *H. contortus* or concurrently.

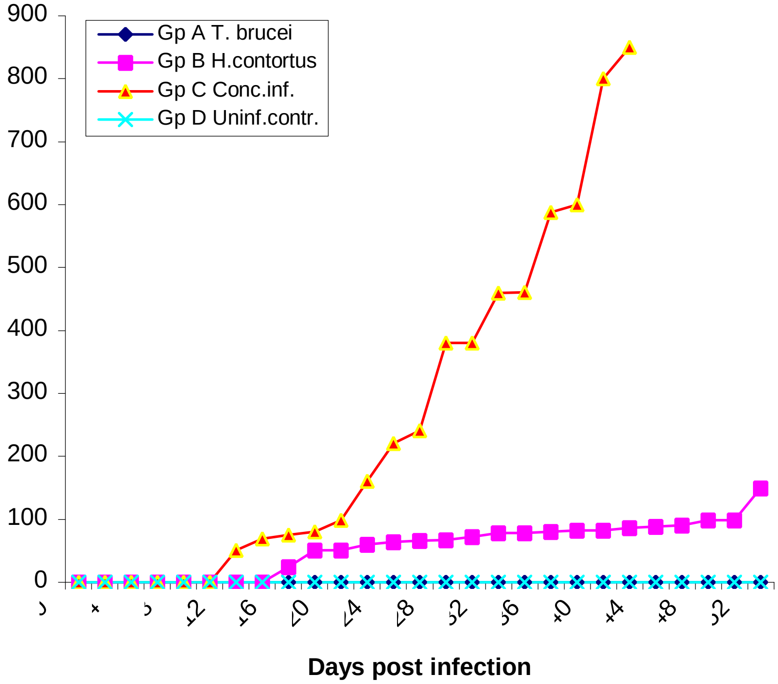

Fig. 2. Mean eggs per gram of faeces (x 10^3) of red fronted gazelles infected singly with *H. contortus* or concurrently.

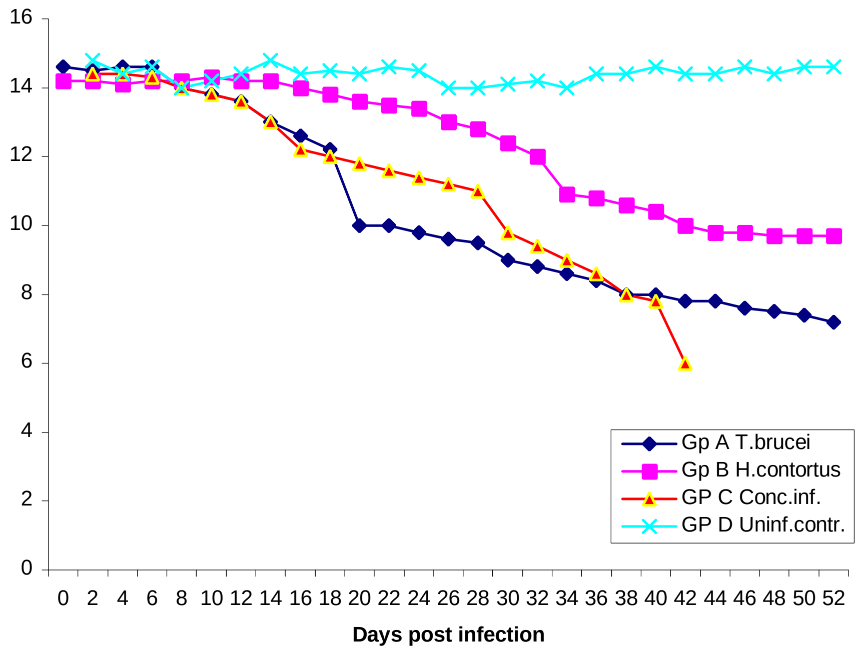

Fig. 3. Mean white blood cell count (x10^3/ul)

of red fronted gazelles

infected either singly with *T.brucei*, *H.contortus* or concurrently.

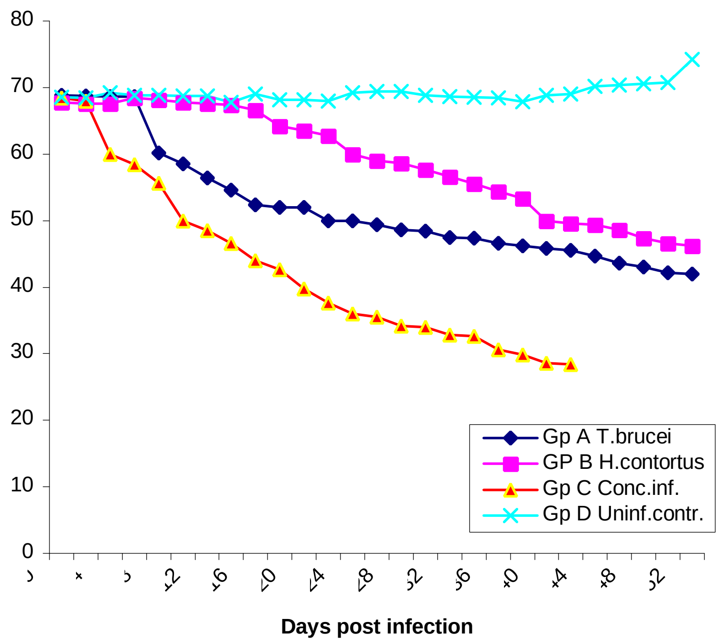

Fig. 4. Mean lymphocyte count (%) of red fronted gazelles

infected either singly with *T. brucei*, *H. contortus* or concurrently.

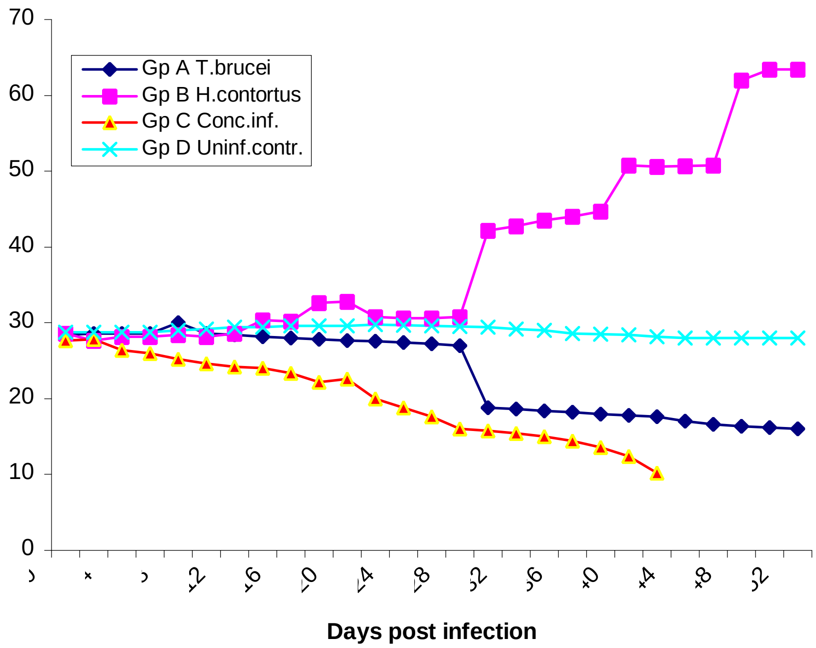

Fig. 5. Mean neutrophil (%) of red fronted gazelles infected either singly with *T.brucei*, *H.contortus* or concurrently.

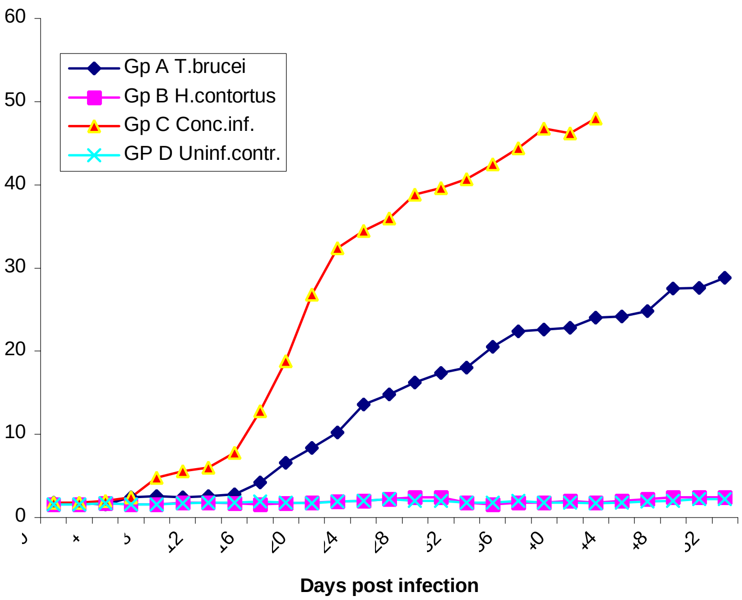

Fig. 6. Mean monocyte (%) of red

fronted gazelles infected

either singly with *T. brucei, H. contortus* or concurrently.

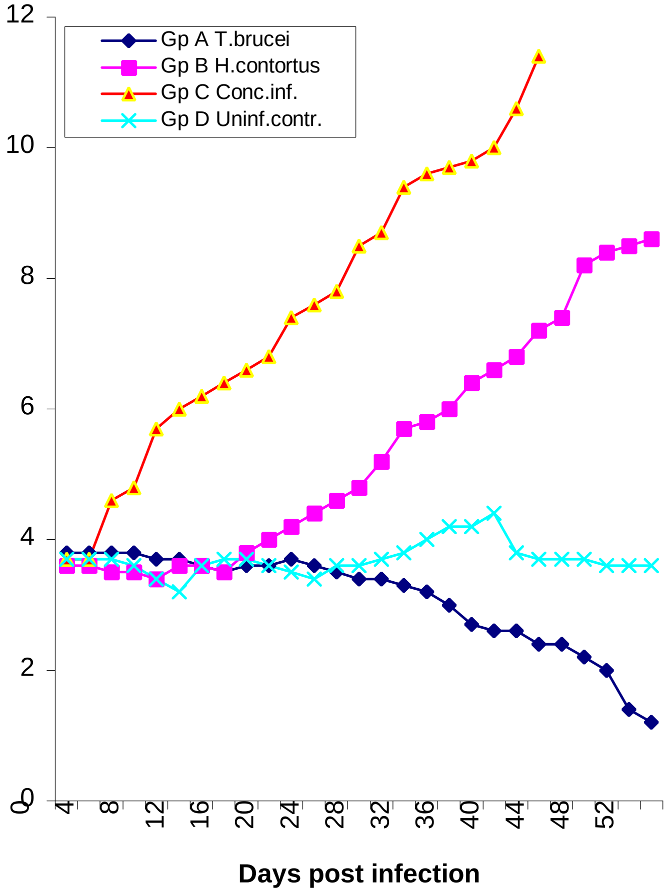

Fig. 7. Eosinophil (%) of red fronted gazelles infected either singly with *T.brucei, H.contortus* or concurrently.