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The relationship between parasitaemia and anaemia in concurrent *Trypanosoma brucei* and *Haemonchus contortus* infections in red fronted gazelles (*Gazella rufifrons*)

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ABSTRACT

The relationship between parasitaemia and anaemia was investigated in red fronted gazelles (*Gazella rufifrons*) experimentally infected either singly with *Trypanosoma brucei*, *Haemonchus contortus*, or concurrently with both parasites. The infections were found to be more virulent in the gazelles with dual infection as compared to those with single infections. The pre-patent periods for *Trypanosoma brucei* and *Haemonchus contortus* in the dual infection were 2-4 and 6-7 days as against 7-8 and 16-17 days during single infections respectively. There was a significant decline ($P<0.05$) in packed cell volume (PCV), red blood cell counts (RBC) and haemoglobin concentrations (Hb), which were more marked in those with the dual infection. Similarly, the erythrocyte sedimentation rate (ESR) was significantly ($P<0.05$) elevated in red fronted gazelles (*Gazella rufifrons*) with the dual infection. From the foregoing we found that an inverse relationship existed between parasitaemia and anaemia in the red fronted gazelles. The effects were however more precipitous in those concurrently infected with both parasites than in those with a single infection of either of the parasites.

Key words: anaemia, parasitaemia, concurrent infection, trypanosomosis, haemonchosis, red fronted gazelles (*Gazella rufifrons*)

Introduction

Grazing animals are usually exposed to concurrent infections and the presence of one parasite may affect other parasites within the same host system (NWOSU et al., 2006).

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Several authors (GRIFFITH et al., 1981; NWOSU et al., 2001; NWOSU et al., 2006) have demonstrated that concurrent *Trypanosoma congolence* infection rendered goats normally resistant to *Haemonchus contortus* more susceptible to the nematode and resulted in more severe pathological effects.

The red fronted gazelle is semi-domesticated in the arid zone of Northeastern Nigeria. It has a high fecundity rate and is reared alongside sheep and goats as a source of lean meat. Recently outbreaks of trypanosomosis due to *Trypanosoma brucei* infection in red fronted gazelles (*Gazella rufifrons*) primarily associated with stress in Maiduguri and Abuja zoos respectively, were observed and reported (MBAYA, 2007). All the gazelles examined during the outbreaks, were found to harbour concurrent infection with *Trypanosoma brucei* and *Trichostrongyles*. Further investigations however, revealed that *Haemonchus contortus* was the predominant nematode in the infection (MBAYA, 2007; MBAYA and ALIYU, 2007). There is however a paucity of such information regarding experimental trypanosomosis and haemonchosis in wild ungulates. This experimental study was therefore undertaken for the first time to evaluate the relationship between parasitaemia and anaemia in a concurrent *Trypanosoma brucei* and *Haemonchus contortus* infection in red fronted gazelles (*Gazella rufifrons*), since both parasites often occur together in the field.

Materials and methods

Experimental animals. Following authorization by the Ministry of the 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 from the Sambisa Game Reserve located in the semi-arid region of north-eastern Nigeria using standard capture techniques (MBAYA, 2004). 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 helminths 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 in the study, were 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) and stabilized by four passages in rats before storage in liquid nitrogen. The stabilates were passaged twice in rats and then transferred into donor Sahel goats which were previously treated against haemo, endo and ectoparasites.

Source of Haemonchus contortus. Gravid adult *Haemonchus contortus* females were collected fresh from the abomasums of goats after slaughter and eviseration 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 and cultured for 10 days at (35 ^{0}C). *Haemonchus contortus* larvae were isolated by the Baerman technique (HANSEN and PERRY, 1994). 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 randomly divided into four groups of five. Group A was infected via the jugular vein with 0.5 mL of blood from the donors diluted with phosphate buffered glucose saline solution (pH 7.4) containing 1.6×10^6 *Trypanosoma brucei*. Group B, was infected orally with 5,000 infective larvae of *Haemonchus contortus* using stomach tube, Group C was infected concurrently with both parasites while Group D served as the uninfected control.

Monitoring of parasitaemia. Parasitaemia due to *Trypanosoma brucei* was first detected by the wet mount and buffy coat dark phase contrast microscopy method (MURRAY et al., 1983) while counts were estimated by the rapid matching technique of HERBERT and LUMSDEN (1976).

Haematological examination. Blood samples were routinely collected every other day via the jugular vein. Packed cell volume (PCV) was determined using the microhaematocrit method, red blood cell (RBC) by the haemocytometer method and haemoglobin (Hb) concentration by the Sahli's method (SHALM et al., 1995). The erythrocyte sedimentation rate (ESR) was determined by the wintrobe method (COLES, 1980).

Statistical analysis. Data obtained from the study were summarized as means ± standard deviation and the differences between the means determined at the 5% level of significance using the analysis of variance (ANONYMOUS, 2000).

Results

Following infection, the prepatent period was 6 to 8 days post infection for gazelles infected singly with *Trypanosoma brucei* and 2 to 4 days for those concurrently infected. Two successive peaks with counts of 250×10^6/μL and 500×10^6/μL were encountered in the first wave and 500.8×10^6/μL and 500.8×10^6/μL for the second waves in those singly infected with *Trypanosoma brucei* and those concurrently infected with both parasites respectively (Fig. 1).

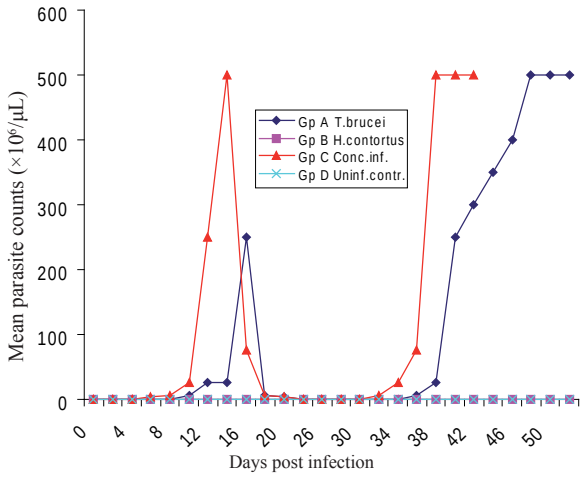

Fig. 1. Parasite counts (×10⁶/µL) of red fronted gazelles (*Gazella rufifrons*) infected singly with *T. brucei*, *H. contortus* or concurrently with both parasites

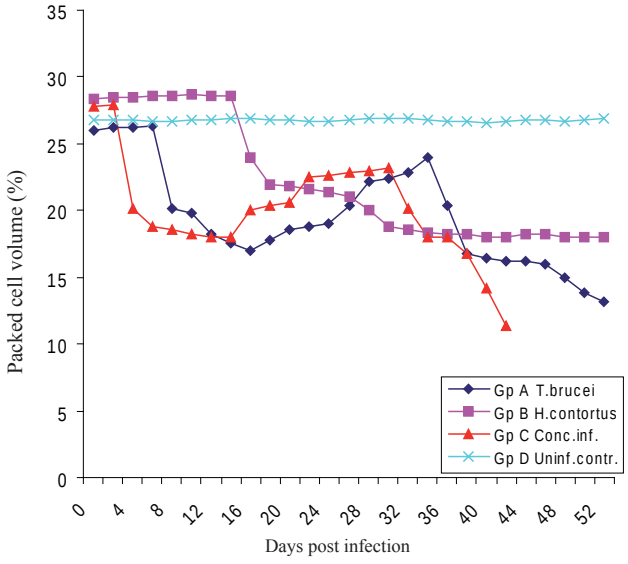

Fig. 2. Packed cell volume changes (%) of red fronted gazelles (*Gazella rufifrons*) infected singly with *T. brucei*, *H. contortus* or concurrently with both parasites

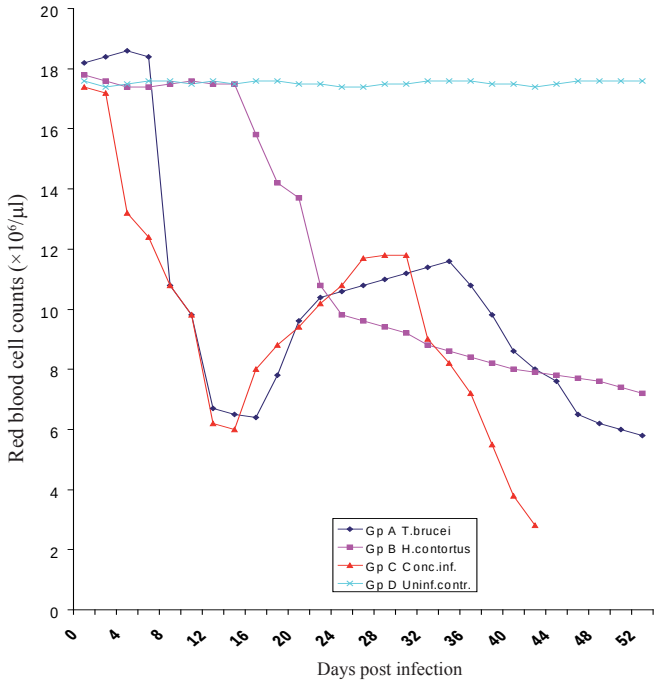

Fig. 3. Red blood cell counts (×10⁶/µl) of red fronted gazelles (*Gazella rufifrons*) infected singly with *T. brucei*, *H. contortus* or concurrently with both parasites

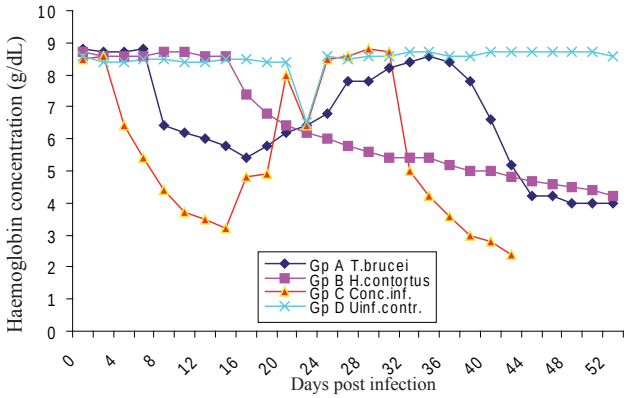

Fig. 4. Haemoglobin concentration (g/dL) of red fronted gazelles (*Gazella rufifrons*) infected singly with *T. brucei*, *H. contortus* or concurrently with both parasites

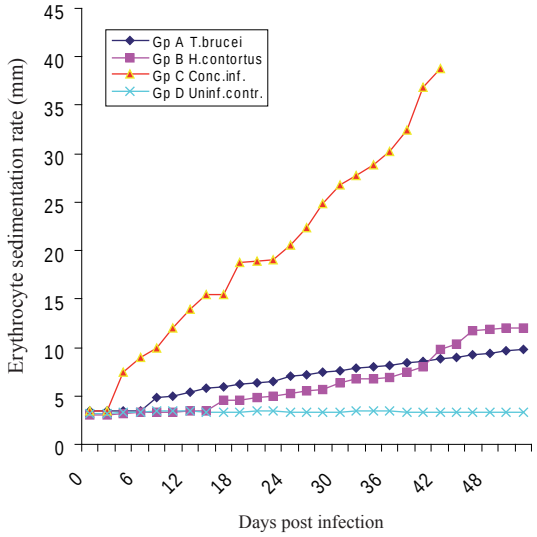

Fig. 5. Erythrocyte sedimentation rate (mm) of red fronted gazelles (*Gazella rufifrons*) infected singly with *T. brucei, H. contortus* or concurrently with both parasites

The packed cell volume (PCV), red blood cell counts (RBC) and haemoglobin concentrations (Hb) experienced sharp falls corresponding to the waves of parasitaemia for gazelles infected either singly with *Trypanosoma brucei* or concurrently with both parasites (Fig. 2, 3 and 4). Similarly, the gazelles infected singly with *Haemonchus contortus* experienced a decline in all red cell indices without the effect of *Trypanosoma brucei*. The decline in the red cell indices was however most pronounced in gazelles with the concurrent infections where values for packed cell volume (PCV), red blood cell counts (RBC) and haemoglobin concentration (Hb) dropped to 18.0 ± 2.12, 6.0 ± 1.22 and 3.2 ± 0.89 respectively. The decline in these values was gradual in the subacute phase but were sharp again at the second wave of parasitaemia, with these values dropping as low as 11.4 ± 1.69, 2.8 ± 0.89 and 2.4 ± 0.77 for the respective indices when all concurrently infected gazelles died by day 42 post infection. On the other hand, those singly infected with *Haemonchus contortus*, the decline in the red cell indices did not become apparent until day 16 post infection.

Immediately after infection, the erythrocyte sedimentation rate (ESR) in all infected groups became significantly ($P<0.05$) elevated and continued to rise unabatedly to peak

values of 9.8 ± 1.57 for gazelles infected singly with *Trypanosoma brucei*, 12.0 ± 1.73 for those infected singly with *Haemonchus contortus* and 38.8 ± 3.11 for those with the dual infection in relation to the values of the uninfected control (Fig. 5). Comparatively, the increase in erythrocyte sedimentation rate (ESR) started much earlier and was significantly ($P<0.05$) higher in gazelles with the concurrent infection.

Discussion

Successive waves of parasitaemia were observed during the single *Trypanosoma brucei* and during concurrent infections with *Haemonchus contortus*. It is a known characteristic of the salivarian trypanosomes to show antigenic variation, which might have been responsible for the successive waves of parasitaemia encountered during the study (SHAPIRO and PEARSON, 1986). It is also believed that wild animals in general are trypanotolerant. The ability of wild animals therefore to limit anaemia or the peak and number of each wave of parastiaemia or to even manifest self-cure, is the measure of its trypanotolerance (MURRAY et al., 1990). Reports, however, of outbreaks due to trypanosomosis in various wild animal species have been reported in captivity elsewhere in the world (MARIE, 1998; PARIJA and BHATTACHARYA, 2005) and recently in red fronted gazelles (*Gazella rufifrons*) in zoological gardens in Nigeria (MBAYA, 2007).

In this present study, where a standard infection was administered and different pre-patent periods were observed with either acute or sub-acute infection, the initial parasite replication rates were very fast among gazelles with concurrent infection, resulting in a shorter pre-patent period. The shorter pre-patent periods in the concurrently infected gazelles might have occurred due to the additive effects of one parasite, making the host environment more favourable for the second parasite or the suppression of the immune responses of the host to one parasite by another (NWOSU, 2001). This might have further been compounded due to the stress of captivity. The break down of trypanotolerance in red fronted gazelle (*Gazella rufifrons*) has been observed to be associated with the immuno suppressive effect of high blood cortisol output, induced by the stress of captivity (MBAYA, 2007).

The anaemia in the gazelles was manifested by a drastic reduction beyond pre-infection values of all the red cell indices during the first wave of parasitaemia for gazelles infected either singly with *Trypanosomas brucei* or concurrently with both parasites. The anaemia in trypanosomosis usually starts early during the first wave of parasitaemia (MBAYA et al., 2007). In this experiment, the development and degree of anaemia varied in intensity in various groups. Those concurrently infected had earlier onset and more severe anaemia, followed by those infected singly with *Trypanosoma brucei*, then *Haemonchus contortus* in that order. Although the exact cause of each of these is unknown, the fact that the PCV, RBC, and Hb values decreased sharply in periods of high parasitaemia, but maintained

a gradual decrease during the period of low parasitaemia, shows a direct relationship between anaemia and parasitaemia. Similar fluctuations in erythrocyte values have been demonstrated in *Trypanosoma brucei* infection of dogs (NWOSU and IKEME, 1992).

The development of the anaemia with such intensity in the concurrently infected gazelles might be cumulative, probably due to the complexity of the mechanisms of red cell injury in trypanosomosis (IGBOKWE, 1994) coupled with the haematophagous activity of *Haemonchus contortus* (GRIFFITH et al., 1981). Such synergistic interactions have been observed in concurrent *Trypanosoma congolense* and *Haemonchus contortus* infection in goats (GRIFFITH et al., 1981). The development of anaemia in animal trypanosomosis in general is haemolytic in nature with the etiology complex and multifactorial, which includes the expanded and active mononuclear phagocytic system (IGBOKWE, 1994). Erythrophagocytosis is sometimes caused by the mononuclear phagocytic system (MPS), which developed soon after infection and continued thereafter in both the acute and sub-acute diseases. The activation of this system might necessitate an increased demand on the system to remove antigen antibody complex, trypanosomes, dead blood and tissues cells, and to participate in immune responses. It is also likely that the synergistic effects of haemonchosis and trypanosomosis was responsible for the significantly higher erythrocyte sedimentation rate (ESR) recorded in the concurrently infected gazelles than in those suffering from single infections. High erythrocyte sedimentation rates have been reported to be associated with severe tissue damage and necrosis (COLES, 1980; NWOSU et al., 2006).

In conclusion, the results showed that parasitaemia produced more severe anaemia in the concurrently infected gazelles than in those with single infections. It is therefore recommended that routine screening and treatment for both parasites be carried out when they are subjected to captivity in endemic areas.

Acknowledgements

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MBAYA, A. W., M. M. ALIYU, C. O. NWOSU, T. EGBE-NWIYI: Odnos između parazitemije i anemije pri istodobnoj infekciji vrtušcem *Trypanosoma brucei* i oblićem *Haemonchus contortus* u crvenočele gazele (*Gazella rufifrons*). Vet. arhiv 79, 451-460, 2009.

SAŽETAK

Odnos između parazitemije i anemije istražen je u crvenočele gazele (*Gazella rufifrons*) pokusno invadirane zasebno vrtušcem *Trypanosoma brucei* i oblićem *Haemonchus contortus* ili istodobno obim parazitama. Zaraza je bila mnogo teža u gazela s dvojnom invazijom u odnosu na one invadirane jednom vrstom parazita. Prepatentni period kod dvojne invazije iznosio je 2-4 dana za vrstu *Trypanosoma brucei*, a 6-7 dana za *Haemonchus contortus*, dok je kod pojedinačne invazije prepatentni period za *Trypanosoma brucei* bio 7-8, a za *Haemonchus contortus* 16-17 dana. Ustanovljen je značajni pad vrijednosti volumena koncentriranih stanica (hematokrita) ($P<0,05$), broja crvenih krvnih stanica i koncentracije hemoglobina što je bilo izraženije kod dvojne infekcije. Sedimentacija eritrocita je također bila značajno ($P<0,05$) povećana kod dvojne invazije. Iz navedenoga se vidi da postoji obrnuti odnos između parazitemije i anemije. Učinci su ipak bili mnogo jače izraženi u gazela istodobno invadiranih obim parazitima nego u onih invadiranih jednom vrstom parazita.

Ključne riječi: anemija, parazitemija, istodobna invazija, tripanosomoza, hemonkoza, crvenočela gazela, *Gazella rufifrons*