

## Changes in Some Haematological Values Associated with Mixed *Trypanosoma congolense* and *Haemonchus contortus* Infection in Yankassa Sheep

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**Abstract:** An experimental study on joint infection with *Trypanosoma congolense* (Tc) and *Haemonchus contortus* (Hc) was conducted in 30 female Yankassa sheep aged between 12 and 25 months, divided into 5 groups of 6 animals (TcHc, HcTc, Hc, Tc and control). Two groups received a single infection with either *H. contortus* or *T. congolense* and two other groups were infected with *T. congolense* followed by *H. contortus* (TcHc) in one and *H. contortus* followed by *T. congolense* (HcTc) in the other. One group was kept as uninfected control. All the trypanosome-infected groups (Tc, TcHc, HcTc) showed different prepatent periods, the Tc group was patent at day 10 post-infection (pi), while TcHc and HcTc were patent at day 5 and 7 pi, respectively. High mean parasitaemia was observed during the acute phase of the infection in the trypanosome infected groups, which declined during the chronic phase giving mean parasitaemic scores of  $3.2 \pm 0.1$  and  $0.3 \pm 0.1$ . There was a rapid fall in the Packed Cell Volume (PCV) of the TcHc group, which reaches a minimum decline at day 25 pi. It was also observed that higher faecal egg count corresponded to decrease in PCV. All the *Haemonchus* infected groups developed higher eosinophils values, which was indicative of the activities of tissue invading helminthes.

**Key words:** *Trypanosoma congolense*, *Haemonchus contortus*, haematology, Yankassa sheep, infection, Nigeria

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### INTRODUCTION

Both haemonchosis and trypanosomosis cause anaemia in sheep (Gossens *et al.*, 1997). In the case of *Haemonchus*, the anaemia is due to the haematophagous activities of the nematode and the accompanying hemorrhage. In trypanosomosis, the anaemia is primarily a result of the trypanosomes damaging the red cell membranes which results in the exposure of antibody binding sites on their surfaces which are normally hidden. Such sites are recognized by circulating antibodies whose function is to bind damaged red cells, thus facilitating the removal of old blood cells by macrophages (Horst, 1996). In both infections, there is evidence to suggest that these features are initially accompanied by marked stimulation of erythropoietic activity (Katunguka-Rwakishaya *et al.*, 1997). *Haemonchus contortus* is probably the only nematode parasite of sheep and goats that can be accurately diagnosed without the aid of laboratory testing because signs of acute anaemia are obvious if other less common conditions causing anemia are discounted

(Okaiyeto, 2006). This study highlights some haematological changes associated with concurrent infection of *Trypanosoma congolense* and *Haemonchus contortus* in Yankassa sheep.

### MATERIALS AND METHODS

**Animals:** Thirty female Yankassa sheep aged between 12-25 months and having approximately the same body weight and health status were used for the study. The sheep were housed in fly and tick-proof pens in the Department of Veterinary Parasitology and Entomology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria. All selected sheep were screened for haemoparasites along with other internal and external parasitic infections and infestations. Irrespective of infection or otherwise, all the animals were prophylactically treated with 2.5% suspension of albendazole (SAM Pharm, Nigeria Ltd.) at a dose rate of  $7.5 \text{ mg kg}^{-1}$  body weight given per os. Also, the animals were treated with diminazine aceturate at  $3.5 \text{ mg kg}^{-1}$  body

Table 1: Experimental protocol for single and mixed infection of *T. congolense* and *H. contortus*

Groups	No. of animals infected	Day 0	Day 7
A	6	1500 L <sub>3</sub> <i>H. contortus</i> /animal orally	-
B	6	10 <sup>6</sup> <i>T. congolense</i> /animal (iv)	-
C	6	10 <sup>6</sup> <i>T. congolense</i> /animal (iv)	1500L <sub>3</sub> of <i>H. contortus</i>
D	6	1500L <sub>3</sub> of <i>H. contortus</i> /animal orally	10 <sup>6</sup> <i>T. congolense</i> per animal (iv)
E (control)	6	Control	Control

L<sub>3</sub> = Infective Larvae; iv = Intravenous

weight (Hoechst, Germany) against trypanosomosis and with long acting oxytetracycline (Pfizer Products Ltd, Ikeja, Nigeria) at 20 mg kg<sup>-1</sup> body weight intramuscularly against *Anaplasma ovis*. They were also vaccinated against Peste des Petit Ruminantium (PPR). Ectoparasite control was carried out by spraying the sheep with a solution of Asuntol (Coumaphos) at a concentration of 1 mL L<sup>-1</sup> of water (Bayer AG, Germany). They were ear tagged, kept in different groups including control and maintained in complete hygienic conditions. They were provided with groundnut and bean crop residues, Cotton seed cake, wheat bran and a salt-lick and water was provided *ad libitum*.

**Experimental design:** The 30 experimental animals were divided into five groups: A, B, C and D. Groups A, B, C and D consisted of 6 animals each, while Group E (control) had 6 animals (Table 1).

**Infection with parasites:** Animals in groups A and B were singly infected with *Haemonchus contortus* and *Trypanosoma congolense*, respectively. Animals in Group C were infected with *T. congolense* at day 0 and 7, they were infected with *H. contortus*. While the group D animals were infected with *H. contortus* at day 0 and at day 7, they were infected with *T. congolense* Group E animals serve as control.

The laboratory strain of *T. congolense* was obtained from the National Institute of Trypanosomosis Research (NITR) Vom, Plateau state, Nigeria and maintained in laboratory animals (mice) for 5 days. At the peak of parasitaemia, the trypanosomes were harvested and inoculated into a donor sheep, while in the donor sheep, at high level of parasitaemia, 1×10<sup>6</sup> trypanosomes were from the jugular vein and inoculated into each of the experimental sheep intravenously. Hundreds of adult female *H. contortus* were recovered from ovine abomasum purchased from the abattoir. The tissues were crushed gently in the mortar and mixed with sterile bovine faeces and then incubated for 7 days at 23-27°C. The third stage Larvae (L<sub>3</sub>) were recovered on day 7 and the concentration per 100 µL determined. Each of the experimental animals was given 1500 L<sub>3</sub> per os.

**Sample collection:** For parasite identification and estimation, 10 mL of blood were collected via the jugular

vein of each sheep twice a week at three day intervals for 2 weeks. Thereafter blood samples were obtained once a week for the rest of the experimental period. Six milliliters of the collected blood were placed in clean Vacutainers without anticoagulant for serum extraction and the remaining 4 mL placed in vacutainers containing anticoagulant Ethylene Diamino Tetracetate (EDTA) for haematological analysis. From day 2 of infection 2 g of faecal sample was collected via the rectum in clean polythene bag twice a week at 2 days interval for the period of the experiment.

**Sample analysis:** Level of parasitaemia, Pack Cell Volume (PCV) and Total Protein (TP) were estimated using microhaematocrit (HCT) and the refractometric method, respectively. The faecal samples collected were processed by the simple floatation method as described by Anonymous (1977) and then examined for helminthes eggs under x 10 objective.

Statistical analysis was carried out using SAS<sup>R</sup> (version 6.11) statistical package (Schlotzhauer and Littell, 1989) and the value of p<0.05 was considered significant.

## RESULTS

All the sheep in the infected groups A, B, C and D developed varying degree of anaemia. In *H. contortus* infected animals, a slight fall in mean pack cell volume was observed from day 25-40 Pi (Fig. 1). Where as in *T. congolense* infected animals, a decreased in PCV started from day 15-50 Pi. In group C (Tc+Hc) having dual infection, the animals showed marked decrease in PCV from day 8-50 Pi.

Similarly group D (Hc+Tc) where L<sub>3</sub> (*H. contortus*) larvae preceded the *T. congolense* infection 1 week after the PCV decreased from day 15-50 Pi. When the mean PCV of group C (Tc+Hc) was compared with the mean PCV of group D (Hc+Tc), there was significant differences (p<0.005). All the *Haemonchus* infected groups (Hc, Tc+Hc and Hc+Tc) showed marked eosinophilia, the value observed showed significant differences (p<0.05), when compare with that of control (Fig. 2). The trypanosomes infected groups (B, C and D) showed different prepatent periods. A parasitaemia in the Tc+Hc group was present on day five, while in the Hc+Tc group a parasitaemia was present on Day 7 Pi.

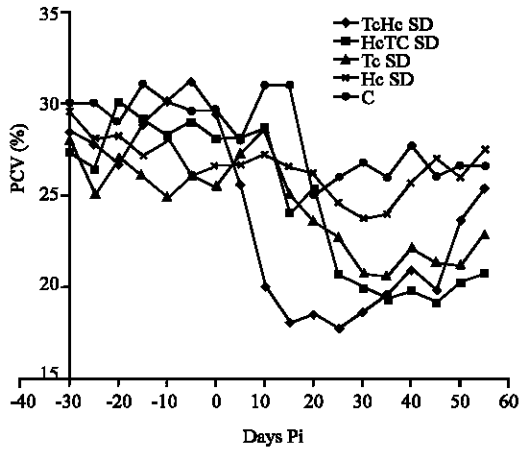


Fig. 1: Mean PCV of Yankassa sheep with a single or mixed infection of *T. congolense* and *H. contortus*

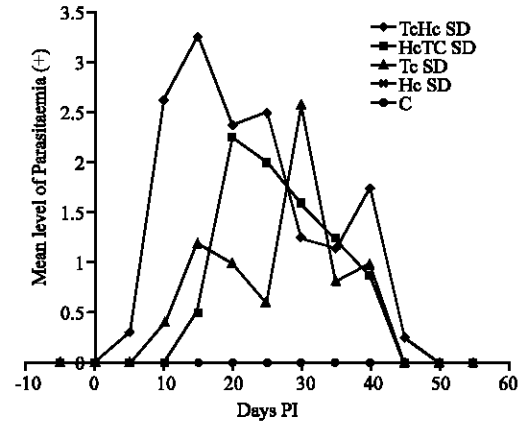


Fig. 3: Mean parasitaemia of single and mixed infection of *T. congolense* and *H. contortus* in Yankassa sheep

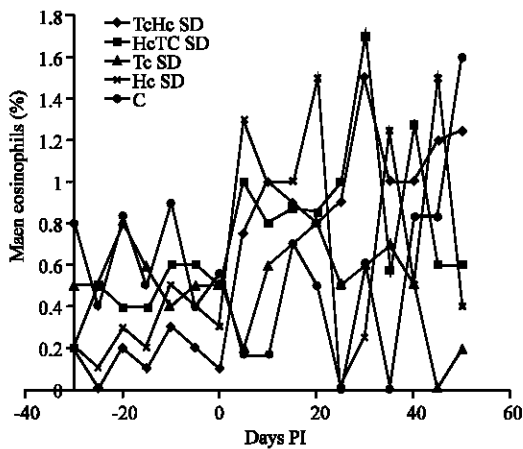


Fig. 2: Mean Eosinophil Count of Yankassa sheep with a single or mixed infection of *T. congolense* and *H. contortus*

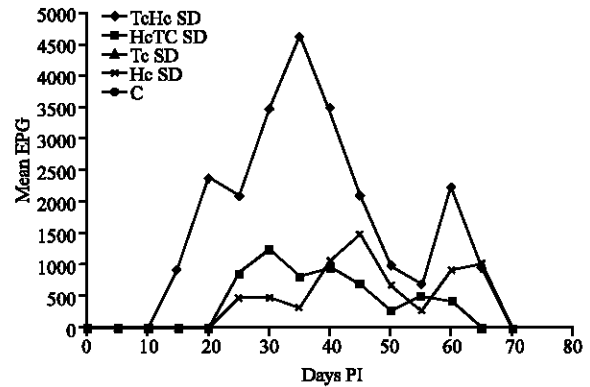


Fig. 4: Mean EPG of Yankassa sheep with a single or mixed infection of *T. congolense* and *H. contortus*

The *Tc* group had a prepatent period of 10 days post infection. The mean parasitaemia remained high during the initial phase of the infection in the Tc+Hc and Hc+Tc infected groups, given a mean parasitaemic score of  $3.2 \pm 0.1$  and  $2.2 \pm 0.3$ , respectively on day 12 Pi but this declined to  $0.3 \pm 0.1$  and zero level, respectively for the Tc+Hc and Hc+Tc group on day 45 post-infection (Fig. 3). All the sheep infected with *H. contortus* larvae (group A) developed a patent infection by day 25 Pi. The sheep in group C (Tc+Hc) developed a patent infection on day 15 post infection, whereas sheep in group D (Hc+Tc) started discharging eggs in their faeces on day 25 post-infection. A mean weekly egg per gram of faeces (epg) of  $4635 \pm 1.5$  was observed in sheep infected with Tc+Hc group on day 34 post-infection and

was significantly higher ( $p < 0.05$ ) when compared with group D (Hc+Tc) infected animals (Fig. 4). Thereafter, the egg in group C declined to  $960 \pm 1.5$  epg on day 65 pi and was zero by day 70.

## DISCUSSION

The present study investigated some haematological changes associated with a mixed infection of *Trypanosoma congolense* and *Haemonchus contortus* in sheep. It was observed that all the sheep in the infected groups (A-D) developed varying levels of anaemia (Fig. 1). Severe anaemia was observed in group C (Tc+Hc) with a dual infection, as a result of both haematophagous activities of the *Haemonchus* parasites (Egbe-Nwiyi *et al.*, 1999) and the destruction of the Red Blood Cells (RBC) by the trypanosomes. In the present study, a significant relationship was observed between

the mean Packed Cell Volume (PCV) and mean intensity of parasitaemia that means the higher the parasitaemia the higher the level of anemia. However, when all the values of the PCV of the four groups were compared, there was no statistical differences. A higher level of parasitaemia was observed. Similar observations have been reported in goats with mixed infection of *T. congolense* and *H. contortus* (Griffin *et al.*, 1981). However, Paling *et al.* (1987) reported that in *T. congolense* infected N'dama cattle that although, the intensities of parasitaemia and anaemia were related, the relationship was not strictly quantitative. It was also observed in this study that higher egg per gram of faeces (epg) in the *H. contortus* infected sheep corresponded with a decrease in PCV suggesting that the heavy worm load might have contributed to the development of anemia. The higher egg count observed in (Tc+Hc) could have been attributed to the immunosuppressive effect of the *T. congolense* infection resulted in a reduction in the resistance of the affected sheep to the *H. contortus* infection. This finding is similar to the report of Dwinger *et al.* (1994) who observed higher egg counts in trypanosome-infected N'dama cattle, while Baker (1995) suggested that faecal egg output depends not only on the percentage of females established but also on their fecundity, which in turn, depends on the population pressure, the season and the resistance of the breed.

Eosinophilia was observed in the three groups of Yankassa sheep that received a *H. contortus* infection (single or mixed). Increased eosinophilia is indicative of infection by tissue invading helminth (Soulsby, 1982). Since the current study was conducted during the dry season, it is not unlikely that some of the *H. contortus* larvae might have undergone arrested development (Ogunsisi and Eysker, 1979) thus triggering an eosinophilic response through the activation of T-helper cells (Perez *et al.*, 2003). Egbe-Nwiyi *et al.* (1999) reported higher eosinophils in small ruminants infected with gastrointestinal parasites.

### CONCLUSION

The mean Packed Cell Volume (PCV) declined more rapidly in the *T. congolense/H. contortus* infected group and reached a minimum on day 25. Also observed in the same group in this study was a higher mean faecal egg count which corresponded with a decrease in the mean PCV, which is a measure of the degree of anemia. The development of a higher epg could be attributed to the immunosuppressive effects of the *T. Congolense*

infection in the *T. congolense/H. contortus* infected Yankassa sheep. The presence of a marked eosinophilia is a strong indication of helminth infection, especially infection by tissue invading helminths. One could surmise that in trypanosome- infected animals, when the level of anemia is high it is advisable to examine the faeces for helminthiasis, because this study has shown the immunosuppression due to trypanosomiasis can exacerbate the effects of the nematode which can lead to an increase in the level of anemia.

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