

Full Length Research Paper

# Serum Micromineral Alterations in Sheep Infected with *Trypanosoma congolense*

Neils, J. S.<sup>1</sup>, Joshua, R. A.<sup>2</sup>, Oladusu, L. A.<sup>2</sup>

<sup>1</sup>Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria. Nigeria.

<sup>2</sup>Faculty of Veterinary Medicine, University of Ibadan, Ibadan. Nigeria.

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Twenty (20) Yankassa sheep ages between 12-18 months were infected with fresh stock of *Trypanosoma congolense* isolated from a cow. Animals were grouped into three; groups A and B were infected while group C served as uninfected controls. Samples between the infected and the uninfected controls showed a high significant levels of calcium (Ca) and iron (Fe) ( $P < 0.001$ ) and lower of phosphorus (P) ( $P < 0.001$ ). There was no significant change in the level of copper (Cu) ( $P > 0.05$ ). Generally, values of the contemporaneously uninfected sheep were significantly lower for calcium and iron and higher for phosphorus and copper. Therefore, the increase in concentrations of calcium, iron and phosphorus may suggest that they could have a role in the pathogenesis of trypanosomosis due to *T. congolense*.

**Key words:** Yankassa sheep, microminerals, *T. congolense*, pathogenesis.

## INTRODUCTION

Animal trypanosomoses constitute serious threat to livestock production in Africa, especially in countries where *Glossina* (tsetse flies) are predominantly found. Typical African trypanosomiasis in Nigeria are caused by *Trypanosoma brucei brucei*, *T. congolense*, *T. simae* and *T. vivax*, which infect cattle, sheep, goats, dogs, horses, camels and donkeys (Losos and Ikede, 1972; Oniyah, 1997). Sheep and goats, however, are seldom found infected with trypanosomes under natural conditions. Losos and Ikede (1972) estimated that of all disease due to trypanosomoses in Africa, sheep and goats accounted for 2%, the low incidence rate may be due to their resistance to trypanosomes. However, over time, the prevalence rates have changed to 8.6% for sheep and 8.1% for goats (Oniyah 1997).

It has been estimated that Nigeria has 22.1 million sheep and 35 million goats (Afolayan et al., 2001). The

majority of sheep and goats in Nigeria are found in the Northern rural areas, reared alongside cattle or with sedentary farmers. These small ruminants may therefore serve as alternative to tsetse flies during feeding in an infested area. Clinical manifestations of trypanosomosis in small ruminants are mainly the acute and chronic forms of the disease where anaemia is the cardinal sign (Losos and Ikede, 1972).

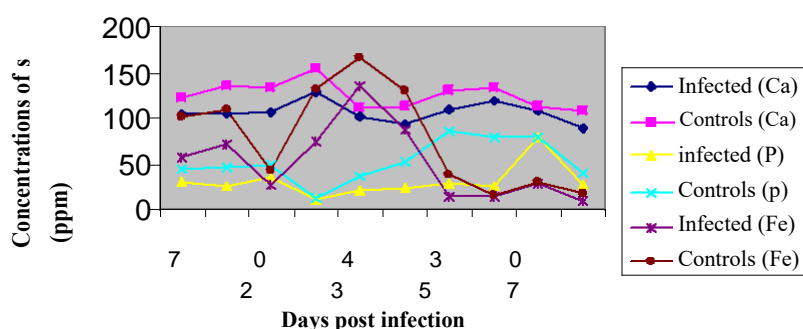
Numerous physiological factors like diseases (such as trypanosomosis) can produce variation in micromineral concentrations in the blood of healthy sheep and cattle (Tartour, 1973; Moodie, 1975). Wellede et al. (1989) showed that serum iron and serum- iron binding capacity for cattle decreased when infected with *T. congolense*. Cattle infected with *T. congolense* showed elevated levels of serum iron (SI), total iron binding capacity (TIBC), plasma iron turn over rates (PITR) and plasma iron clearance (PIC). In treated animals, SI and TIBC falls with the level of SI returned to preinfection level faster than TIBC.

Dargie et al. (1980) reported that, in trypanosomosis due to *T. congolense* in cattle, there was loss of 40-45%

\*Corresponding author. E-mail: [adeolajegede@yahoo.com](mailto:adeolajegede@yahoo.com) Tel: +234-8044 -133649.

**Table 1.** Mean mineral concentrations in sheep infected with *T. congolense*.

Minerals (ppm)		7	13	20	27	34	47	53	59	70	117
		Days post infection									
Calcium	Infected	105.3	104.38	106.25	128.75	101.88	93.13	109.1	119.38	108.13	88.13
	Controls	121.88	135.0	132.8	154.1	110.6	112.3	129.38	133.4	113.1	107.8
Phosphorus	Infected	30.4	25.6	35.0	10.8	20.6	24.3	28.6	26.1	79.0	27.0
	Controls	44.0	46.0	49.3	13.0	36.0	51.63	85.75	80.0	90.2	39.1
Iron	Infected	56.6	71.9	26.25	74.1	135.4	87.8	13.8	13.8	28.7	9.38
	Controls	101.38	108.75	42.5	132.5	166.6	129.38	38.13	15.63	30.63	16.9
Copper	Infected	0.69	0.75	0.38	0.63	0.62	0.75	0.75	1.13	1.0	0.50
	Controls	0.69	0.88	1.1	1.38	1.1	0.82	0.84	1.37	1.07	0.56



**Figure 1.** Mean mineral concentrations in sheep infected with *T. congolense*.

of iron in circulation. However, Sarror (1976) found neither iron nor copper deficiency play any important role in the pathogenesis of anaemia in cattle infected with *T. vivax* and found no change in the levels of these microminerals in the serum of the cattle. Copper level fluctuates but within normal limits (Anosa 1988; Joshua et al., 1994). Kalu et al. (1989) reported that the level of calcium in the serum of goat increased significantly during acute stage of trypanosomosis. After chemotherapy, there were constant decrease levels of calcium, which was similar with the increase in total protein level (Kalu et al., 1987). Anosa (1988) reported a decrease in phosphate levels in cattle infected with *T. congolense*. The depression of calcium observed was thought to be due to thyroid gland damage.

This study was carried out with the view to determine the response of calcium, iron, phosphorus and copper in the plasma of sheep infected with *T. congolense*.

## MATERIALS AND METHODS

### Experimental Design

Twenty (20) Yankassa sheep were used for this study and dental eruption was used for estimation of their ages. They were housed in a fly proof pen and fed with variety of fresh grass cut supplemented

with spent grains daily. *T. congolense* was isolated from a cow and was screened and passaged in mice as described by Lumsden et al. (1973). The parasites were continually maintained in the mice until the donor animal was infected. Sheep were inoculated with *T. congolense* as follows:

**Group A.** There were seven animals in this group and each animal was inoculated with 1 ml of  $10^7$  parasites via the external jugular vein. The animals were treated with Samorin®. The treatment was done on day 25 post infection after the first wave of parasitemia. The drug was given intramuscularly.

**Group B.** Seven animals were inoculated with the same number of parasites as in group A via the external jugular vein. The animals were treated with Berenil®. The treatment was done on day 25 post infection after the first wave of parasitemia. The drug was given intramuscularly.

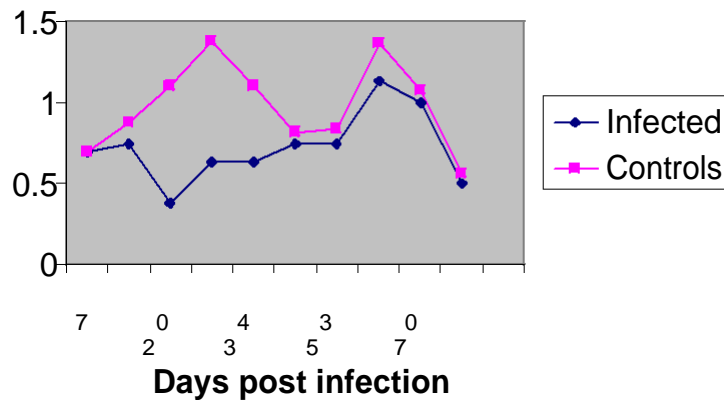
**Group C.** This group of six served as uninfected controls.

### Blood sampling

Parasitological examinations were carried out with 2 ml of blood collected via the external jugular vein in a Bijou bottle with EDTA as the anticoagulant. The bleeding was done twice a week.

### Serum collection

5 ml of blood was collected via the external jugular vein from each animal in the different groups. Samples were collected on the same day with that for parasitological examinations. Blood was collected



**Figure 2.** Mean copper concentrations in sheep infected with *T. congolense*.

in clean dry test tubes and kept at room temperature for a period sufficient to allow separation. Serum was harvested in a sterilized Bijou bottle and stored at  $-20^{\circ}\text{C}$  until required.

#### Micromineral concentrations

The concentrations of calcium, iron and copper were determined using atomic absorption spectrophotometer. For phosphorus, the Auto analyzer II was used. Sera were digested with hyperchloric ( $\text{HClO}_4$ ) and nitric ( $\text{HNO}_3$ ) acids for the determination of calcium, iron, phosphorus (Kalu et al., 1987), while copper was determined as described by Smith et al. (2000).

### RESULTS AND DISCUSSION

The concentration of iron in sera was found to be depressed on day 20 when the parasitaemia was at peak (Table 1, Figure 1). Calcium concentration was, however, found to be high. The depression of these minerals was found to be statistically significant ( $P < 0.001$ ) between days 20 and 59. Higher value of iron obtained on day 34 was significantly higher ( $P < 0.001$ ) than that of day 20. There was varying concentration of phosphorus in the infected and control animals. The highest value was observed on day 70. Copper was found to be consistently at low values in both infected animals and controls (Figure 2).

This investigation has shown that Yankassa sheep are susceptible to freshly isolated *T. congolense* infection. The infectivity could have been enhanced by passaged in mice, as observed Joshua (1990). Finnes (1954) and Losos and Ikede (1972) associated trypanosomoses in cattle, sheep and goats with heavy parasitaemia, though it was not a constant finding. This study has shown that anaemia produced from trypanosome infection in sheep may be related to the level of parasitaemia. However, parasitaemia fluctuated at intervals as described by Joshua (1990).

The concentration of calcium in the infected animals was found to increase and it correlated with high peak of parasitaemia. Chemotherapy did not change any observable value of calcium level. This agrees with

earlier observations made by Godwin (1974) and Kalu et al. (1987). It is obvious that calcium increases in serum but the source remains unclear; it could be that, calcium ions from the extra cellular fluid that binds cell membranes together with ions in serum are partly mobile elevated the concentration (Georgievskii et al, 1982). This needs further investigation. Phosphorus (P) level was also found to fluctuate in similar pattern to that of calcium, but increase in value was significant ( $P < 0.001$ ) between infected and the controls. Phosphorus is found distributed in almost all organs of the body and plays a role in the production of ATP especially in muscles. During the course of infection, there is reduction in the production of ATP, thus the probable increase of P in serum. Anosa (1988) suggested that, calcium in conjunction with phosphorus depressed thyroid cells, but the actual roles of calcium and phosphorus during trypanosomiasis are not yet known.

Iron concentration in this investigation was found to decrease immediately when parasitaemia was detected and continued to decline until chemotherapy. Earlier, Dargie et al. (1979) and Anosa (1988) reported that there was 40-45 % iron loss and accelerated plasma iron turnover uptake in cattle infected with *T. congolense*. Wellede et al. (1989) reported the return of normal level of iron in animals infected with trypanosomes after chemotherapy as also observed in this study. Sarror (1979) reported similar observations, though in cattle. The role of iron in the pathogenesis of trypanosomosis needs to be elucidated. Copper level was found to only moderately fluctuate and there was no significant difference ( $P > 0.05$ ) between the infected and the controls. Sarror (1979) and (Joshua et al., 1994) also observed that there was no change in copper level when cattle were infected with *T. vivax* and sheep with *T. congolense*, respectively.

Although required copper concentration is 30 times lower than iron (Georgievskii et al., 1989), its storage organelles is more and this could be responsible for the stabilization of copper level during *T. congolense* infection.

In Conclusion, *T. congolense* was found to be pathogenic to sheep. The concentrations of Ca, P and Fe fluctuated with peaks of parasitaemia on days 20 and 59, though P and Fe decreased in concentrations but returned to normal levels after chemotherapy. Cu also fluctuated but was within the normal concentration range. The actual roles played by these microminerals in the pathogenesis of trypanosomosis due to *T. congolense* are not clear and therefore may need further investigation.

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