

Influence of *Trypanosoma congolense* infection on some blood inorganic and protein constituents in sheep

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Key words

Sheep - Lamb - *Trypanosoma congolense* - Blood composition - Blood protein - Infection - Scotland.

Summary

The present study was designed to investigate the changes in plasma concentrations of zinc, copper, calcium, magnesium, inorganic phosphate, total protein, albumin, globulin, and serum iron and iron-binding capacity in sheep infected with *Trypanosoma congolense*. It was observed that infection did not have a significant effect on the plasma concentrations of zinc, copper, calcium, magnesium and inorganic phosphate. The serum iron concentrations in infected animals were higher, but not significantly so, than in control animals. Infected animals developed hypoalbuminaemia and hyperglobulinaemia while changes in total protein were not significant. The relevance of these changes to the pathogenesis of *Trypanosoma congolense* infection is discussed.

■ INTRODUCTION

Changes in the concentrations of inorganic constituents in small ruminants infected with trypanosomes have been investigated on few occasions. Most of the reports concern either *Trypanosoma vivax* or *T. brucei* infections in cattle. An increase in the concentrations of serum chloride and calcium in goats infected with *Trypanosoma vivax* (7) and a gradual fall in the concentration of inorganic phosphate in cattle infected with *T. congolense* have been recorded (5). Changes have also been recorded in levels of serum iron, total iron-binding capacity (12) and plasma proteins (7). However, the reports on these plasma constituents have been contradictory and in many cases have been measured at one time point during a course of infection. The present study was designed to follow the changes in the plasma concentrations of zinc, copper, calcium, magnesium, inorganic phosphate, serum iron, total iron-binding capacity, plasma total protein, albumin and globulin during a course of *T. congolense* infection in sheep.

■ MATERIALS AND METHODS

Experimental animals

Four month old male castrate Scottish Blackface lambs were used in the present study. They were purchased from a local hill farm in the West of Scotland and kept in flyproof isolation units at

Glasgow University Veterinary School. Wood shavings were used as bedding.

Experimental design

Twelve animals were randomly divided into two groups of six animals each. One group was infected with 1×10^5 trypanosomes (*Trypanosoma congolense* 1180) in 2 ml of phosphate buffered saline intravenously and the other group served as uninfected controls. *Trypanosoma congolense* 1180 is a cloned derivative of an isolate made in the Serengeti, Tanzania, as described by Nantulya *et al.* (10). The trypanosomes were raised in irradiated mice and harvested at the first peak of rising parasitaemia.

After infection, the animals were bled three times a week for estimation of parasitaemia by the buffy coat method as described by Murray *et al.* (9) and packed cell volume by the capillary tube centrifugation technique, and once a week for biochemical estimations. The animals were monitored for 10 weeks after infection.

Housing and feeding

Animals were kept together in one large pen in a flyproof isolation unit for four weeks before infection. Each lamb received 500 g of concentrate feed per day, hay and water were available *ad libitum*.

Collection of blood samples

Fifteen millilitres of blood were collected once a week from the jugular vein for biochemical analysis as follows: 5 ml of blood was drawn into lithium heparin tubes for measurement of plasma magnesium, inorganic phosphate, total protein, albumin, zinc and copper; 10 ml of blood was also drawn into iron-free tubes without

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anticoagulant. Serum was harvested 24 h later after centrifugation at 700 g for 20 min. Serum was stored in small aliquots at -20°C until required for measurement of serum iron and total iron-binding capacity (TIBC).

Analytical techniques

Plasma zinc, copper, magnesium and calcium were measured using an atomic absorption spectrophotometer (Perkin Ellmer, Beaconsfield, Bucks, England). Commercial kits were used for the assay of total plasma protein and plasma albumin by the Standard Technicon Autoanalyzer (Boehringer Mannheim GMBH, 680 Mannheim 31, Germany). Plasma globulin concentration was recorded as the difference between total plasma protein and albumin.

Inorganic phosphate, serum iron and TIBC were analyzed with commercial kits supplied by Roche Products Ltd (Welwyn Garden City, England), using spectrophotometric methods.

Statistical methods

Results are presented as group means \pm standard error of the mean (SEM). Comparisons between groups were achieved with one way analysis of variance. The statistical analyses were conducted using Minitab (Ryan, Penn State University, USA) and Animal Designs 1 Programme (Data International Services, Glasgow, Scotland) on an IBM computer (PS/2, Model 30). P values less than 0.05 were considered significant.

RESULTS

Haematological, parasitological and clinical observations

Infection caused a significant decrease in mean packed cell volume (PCV) values between 0 and 70 days after infection (DAI). The mean values decreased from $32 \pm 1\%$ at day 0 to $24 \pm 1\%$ at 23 DAI. The values tended to increase but showed another decrease from $29 \pm 1\%$ at 47 DAI to $25 \pm 1\%$ at 63 DAI. The PCV values in control animals varied between $29 \pm 2\%$ and $32 \pm 1\%$ during the course of infection.

Mean corpuscular volumes (MCV) increased following infection from 31.0 ± 0.5 fl at 0 DAI to 37.2 ± 1.4 fl at 75 DAI. The values in control animals fluctuated between 30.4 ± 0.6 and 32.4 ± 0.7 fl. The anaemia was thus characterized as macrocytic and normochromic.

After a prepatent period of seven to nine days, the mean parasitaemia increased in the infected group to reach the first peak 16 DAI. It then fluctuated considerably but showed continually decreasing peaks after 49 days of infection.

Infection did not cause a significant effect on rate of weight gain when compared to control animals. Infected animals showed an increase in mean body temperature from 39.5°C at 0 DAI to 40.3°C by 7 DAI, after which it fluctuated considerably. In control animals, mean values fluctuated between 38.6 and 39.7°C .

Plasma zinc and copper

The concentration of plasma zinc fluctuated considerably in both infected and control animals. However, the concentration was within the ranges of 49.6 - 104.6 $\mu\text{g/l}$. The concentration of plasma copper tended to be higher in infected than in control animals but this difference was not significant. In infected animals, the values fluctuated between 0.51 - 1.0 mg/l , while in control animals the values were 0.39 - 0.81 mg/l .

Plasma calcium

Infection had no effect on the levels of plasma calcium between 0 and 10 weeks after infection. The values in infected and control animals fluctuated between 98.2 and 109.8 mg/l .

Plasma magnesium

The values of plasma magnesium in infected and control animals fluctuated between 17.0 ± 0.73 and 21.6 ± 0.73 mg/l . There was no influence of infection.

Plasma inorganic phosphate

The concentrations of plasma inorganic phosphate did not change with the course of infection. Values fluctuated between 65.4 ± 3.7 and 92.7 ± 3.1 mg/l in both infected and control animals.

Serum iron concentration

The concentrations of serum iron in infected animals decreased from 2.1 ± 0.1 mg/l at 0 DAI to 1.8 ± 0.1 mg/l at 16 DAI, after which it fluctuated. However, it showed a sharp increase between 21 and 35 DAI and between 42 and 56 DAI. The values of serum iron were moderately higher in infected animals than in control ones between 14 and 63 DAI. The mean concentration in control animals showed a downward trend between 21 and 65 DAI. The values in infected animals were not significantly different from those in control animals.

Serum total iron-binding capacity and percentage saturation of transferrin

The values of serum TIBC tended to decrease with progress of infection, but values were not significantly different from those in control animals. There were no significant differences in the levels of unsaturated iron binding capacity and percentage saturation of transferrin between infected and control animals throughout the course of infection.

Total plasma protein

Total plasma protein concentration in infected animals decreased from 64.7 ± 0.9 g/l at 0 DAI to 59.8 ± 2.2 g/l at 21 DAI (figure 1). It then increased steadily to 76.4 ± 3.1 g/l at 42 DAI and later showed a moderate decline. The values in control animals fluctuated between 60.4 ± 1.0 and 65.4 ± 1.3 g/l , and were not significantly different from those in infected animals.

Plasma albumin

Plasma albumin concentration in infected animals decreased significantly from 37.2 ± 0.6 g/l at 0 DAI to 33.0 ± 1.1 g/l at 21 DAI (figure 1). The mean albumin concentration then tended to increase, but dropped again to 32.0 ± 1.0 g/l at 63 DAI. The mean values in control animals fluctuated between 35.8 ± 0.8 and 38.4 ± 0.7 g/l , and were significantly higher than the values in infected animals.

Plasma globulin concentration

The mean plasma globulin concentration showed an upward trend from 27.5 ± 0.8 g/l at 0 DAI to 32.6 ± 2.3 g/l at 42 DAI. Thereafter, it declined to 29.0 ± 1.4 at 63 DAI. The values in control animals varied between 25.8 ± 0.9 and 28.8 ± 0.6 g/l . The values in infected animals were significantly higher than those in control animals between 35 and 70 DAI.

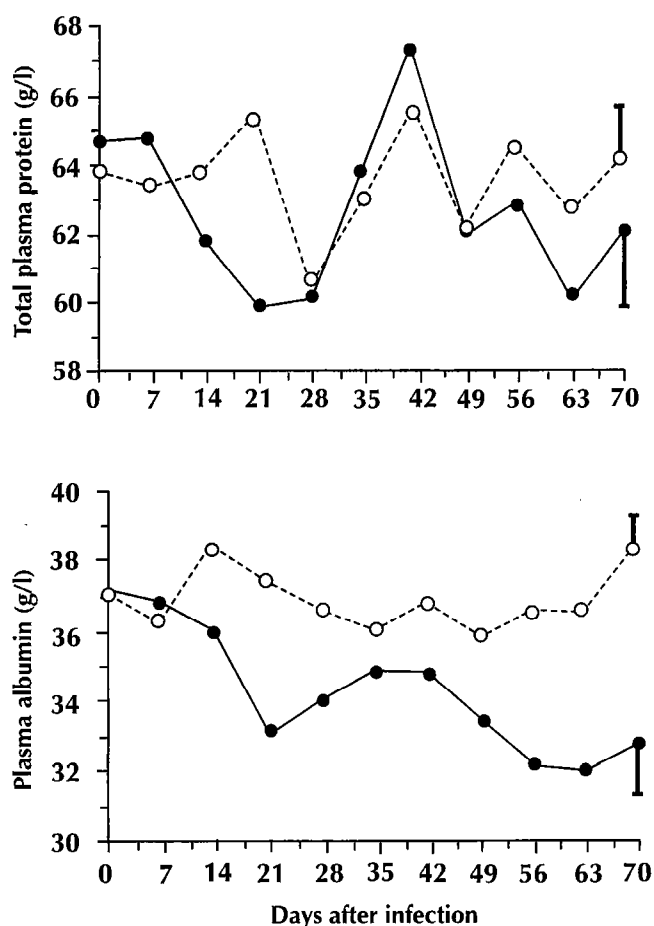


Figure 1: changes in plasma protein and albumin concentrations in sheep infected with *T. congolense*: (●) and in uninfected control sheep: (○)

DISCUSSION

The present study has shown that *Trypanosoma congolense* infection in sheep is not associated with significant changes in the concentrations of plasma zinc, copper, calcium, magnesium and inorganic phosphate. This is in contrast with previous reports, which indicated an increase in the concentration of plasma calcium in West African dwarf goats infected with *Trypanosoma vivax* (7). In goats infected with *T. brucei*, an increase in serum inorganic phosphate between 14 and 84 DAI was observed (6). However, the concentrations of serum copper and magnesium showed a downward trend between 28 and 84 DAI (8). Some authors have reported changes in the plasma concentrations of inorganic phosphate in cattle infected with trypanosomes (5) while others (4) did not. In another study of cattle infected with *T. brucei*, it was found that the concentration of serum magnesium dropped while the concentrations of serum phosphorus did not change with the progress of infection (14). It is possible that these contradictory reports could be related to differences in the trypanosome species or even differences in hosts.

In the present study, the concentrations of serum iron fluctuated considerably in infected animals but were higher than in control animals between 14 and 63 DAI. Serum iron concentrations displayed a sharp increase between 21 and 35 DAI and between 42 and 50 DAI. The sharp increases in serum iron concentrations observed in the present study corresponded with periods of marked decreases in packed cell volumes and red blood cell counts. It is possible that this increase in serum iron concentrations

was associated with intravascular haemolysis, which has been reported to occur particularly in the early stages of trypanosomiasis (3). The bulk of mammalian iron is in haemoglobin and in the liver with lesser amounts in muscles as myoglobin. Since all iron exchanges occur in the plasma, haemolysis would lead to an increase of serum iron.

The concentrations of serum TIBC in infected and control animals were similar in this study. This is in contrast with the observations made in cattle infected with *T. congolense* (12, 14). Serum TIBC is an indirect measure of transferrin concentration, which is the carrier of iron for haemoglobin synthesis (13). A drop in these values may suggest a degree of impaired bone marrow function, which may in turn contribute to the development of anaemia. Since values in infected and control animals were similar, this may suggest that infected animals, in this study, did not experience impairment of bone marrow function.

Infected animals developed a hypoalbuminaemia and hyperglobulinaemia and the changes in total plasma protein were not significant. These observations are in agreement with previous studies of ovine trypanosomiasis (8, 11). The cause of hypoalbuminaemia could be a result of haemodilution, trypanosomal uptake of albumin-bound fatty acids and lipoproteins or increased catabolism by the host (8). There is evidence to suggest that albumin is required by trypanosomes for growth and multiplication. It is therefore possible that development of high parasitaemia particularly in the early stages of infection may lead to increased utilization of albumin, and this coupled with haemodilution may lead to a reduction in plasma concentration of albumin.

An increase in plasma globulin concentration was observed after four weeks of infection. This has been observed in sheep infected with *T. vivax* (2), and in goats infected with *T. brucei* (6). This increase has been attributed mainly to an increase in IgM and, to a lesser extent, of IgG (2). The degree of hyperglobulinaemia observed in the present study is not as marked as that reported in West African goats infected with *T. brucei* (6). It is known that *T. brucei* and *T. vivax* are capable of invading tissues while *T. congolense* is predominantly to be found in the vascular system. It is therefore possible that trypanosomes, which invade tissues, are likely to elicit a greater immunologic reaction leading to production of more immunoglobulins than trypanosomes that are restricted to the vascular system.

CONCLUSION

The present study has revealed that *Trypanosoma congolense* infection in sheep results in changes in protein metabolism and iron metabolism, and has no major effects on the concentrations of plasma zinc, copper, calcium, magnesium and inorganic phosphate.

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Résumé

Katunguka-Rwakishaya E. Influence de l'infection à *Trypanosoma congolense* sur quelques constituants protéiques et inorganiques du sang chez le mouton

Cette étude avait pour objet d'évaluer les variations des concentrations plasmatiques de zinc, cuivre, calcium, magnésium, phosphate inorganique, protéines totales, albumine et globuline, de la sidéremie, ainsi que la capacité de fixation du fer chez des moutons infectés par *Trypanosoma congolense*. Les résultats ont montré que l'infection n'avait pas d'effet significatif sur les concentrations plasmatiques de zinc, cuivre, calcium, magnésium et phosphate inorganique. Les sidéremies des animaux infectés étaient plus élevées que celles des animaux témoins, mais pas de manière significative. Les animaux infectés développèrent de l'hypoalbuminémie et de l'hyperglobulinémie, alors que les variations en protéines totales n'étaient pas significatives. La pertinence de ces variations quant au mécanisme pathogénique de l'infection à *Trypanosoma congolense* est discutée.

Mots-clés : Ovin - Agneau - *Trypanosoma congolense* - Composition du sang - Protéine sanguine - Infection - Ecosse.

Resumen

Katunguka-Rwakishaya E. Influencia de la infección por *Trypanosoma congolense* en algunos constituyentes inorgánicos y proteicos de la sangre en ovinos

El presente estudio se diseñó para investigar los cambios en las concentraciones plasmáticas de zinc, cobre, calcio, magnesio, fosfatos inorgánicos, proteína total, albúmina, globulina y hierro sérico, así como la capacidad de captar el hierro en las ovejas infectadas con *Trypanosoma congolense*. Se observó que la infección no tuvo efecto significativo en las concentraciones plasmáticas de zinc, cobre, calcio, magnesio y fosfatos inorgánicos. Las concentraciones séricas de hierro en los animales infectados fueron superiores, pero no en forma significativa, que en los animales control. Los animales infectados desarrollaron hipoalbuminemia e hiperglobulinemia, mientras que no se observaron cambios significativos en la proteína total. Se discute la importancia de estos cambios con respecto a la patogénesis la infección por *Trypanosoma congolense*.

Palabras clave: Ovino - Cordero - *Trypanosoma congolense* - Composición de la sangre - Proteína sanguínea - Infección - Escocia.