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Neuraminidase (Sialidase) Activity and its Role in Development of Anaemia in *Trypanosoma evansi* Infection

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Abstract: Neuraminidase activity was determined during experimental *Trypanosoma evansi* infection in Savannah Brown bucks. There was significant ($p<0.05$) increase in the activity of sialidase on day 7, 9, 21, 23, 27, 33 and 37 post-infection. Increase in sialidase activity coincided with gradual decline in mean erythrocyte surface sialic acid concentrations occuring 5 days post-infection. There were significant difference ($p<0.05$) in mean erythrocyte surface sialic acid between the infected and control groups on day 5 and between days 17 to 27 post-infection. A significant ($p<0.05$) increase in free serum sialic acid concentrations was observed on days 15, 17 and 27 when compared to the control group. All infected bucks developed trypanosomosis, with significant decreases in mean packed cell volume to as low as 19.50±2.12% occurring at day 33 post-infection which was significantly lower than the control value of 26.75±0.96. Mean haemoglobin concentrations also declined in the infected bucks with marked drop of 6.50±0.70 g dL^{-1} on day 33 post-infection and was significantly different ($p<0.05$) from the uninfected (control) group (8.53±0.46 g dL^{-1}). The anaemia caused during infection may be attributable to the activities of the circulating trypanosomes, which produce sialidase (neuraminidase) that resulted in the cleaving off erythrocyte surface sialic acid, rendering such red blood cells more prone to phagocytosis in the reticuloendothelial system.

Key words: Neuraminidase, infection, *Trypanosoma evansi*

INTRODUCTION

Sialidases have been called neuraminidases or Receptor-destroying Enzymes (RDEs) because they were first described for viruses. They are the key enzymes of sialic acid catabolism, hydrolyzing the glycosidic linkage between the sialic acid molecules and the penultimate sugar of the carbohydrate chains of oligosaccharides and glycoconjugates (Traving and Shauer, 1998). The enzymes occur in higher animals of the deuterostomate lineage that possesses the corresponding sialic acid-containing substrates as components of an autonomous sialic acid metabolism. They are also found in different varieties of micro-organisms such as viruses, bacteria and protozoa (Saito and Yu, 1993; Traving and Schauer, 1998).

One of the distinctive features of trypanosomiasis in animals is the development of anaemia (Suliman *et al*., 1997). In some works carried out on African trypanosomiasis anemia occurs due to erythrophagocytosis (Mamo and Homes, 1975; Homes and Jennings, 1976) which may be associated with the development of antigen-antibody complexes (Audu *et al*., 1999). It was also postulated that anaemia may be attributable to the trypanosomes in the blood which produce neuraminidase (sialidase) which in turn cleaves off surface sialic acid of red cells of cattle making them prone to phagocytosis (Esievo *et al*., 1982). Many of the tsetse-borne parasites have been shown to produce this enzyme and has been demonstrated in *T. vivax* in both *in vivo* and *in vitro* studies (Esievo, 1979; Esievo *et al*., 1982; Esievo, 1983).

Therefore this study was designed to investigate the activity neuraminidase during experimental *Trypanosoma evansi* infection of Savannah Brown bucks.

MATERIALS AND METHODS

Experimental animals: Nine Savannah Brown bucks aged between 12 and 18 months were obtained locally and conditioned for a period of 6 weeks in the Department of

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Of the nine bucks five were infected via jugular vein with approximately 3.0×10^6 *T. evansi* obtained from naturally infected camel slaughtered at Kano abattoir. The remaining four bucks served as control.

Sample collection: For one week following the experimental inoculation of the animals, 6 mL of blood was collected daily via jugular venipuncture for 7 days. Subsequently the sampling was done every other day for the remaining part of the experiment. Of the blood samples collected from each animal, 2 mL was placed into a test-tube containing EDTA as anticoagulant for haematological evaluation, 2 mL was placed into another test-tube containing. 0.3 mL of Acid Citrate Dextrose (ACD) anticoagulant for preparation of haemoglobin-free (ghosts) erythrocytes. The remaining 2 mL were placed into another test-tube without anticoagulant for serum extraction. Serum was prepared within one hour by centrifugation of clotted blood and kept frozen at -20°C until required.

Haematological evaluation: Packed Cell Volume (PCV) and haemoglobin concentrations were determined by standard methods (Schalm *et al*., 1975). Parasitaemia level was determined using the scoring method of Paris *et al*. (1982).

Preparation of haemoglobin-free erythrocyte membranes (ghosts): Ghosts were prepared by the method of Dodge *et al*. (1963) on the day of sample collection using 2 mL of blood in 0.3 mL ACD.

Assay of Erythrocyte Surface Sialic acid : Point zero five (0.05) mL of washed erythrocyte ghosts suspension was incubated at 80°C in 0.02 mL of 0.1 N H_2SO_4 for 1 h to liberate surface bound sialic acid (Warren, 1959, 1963). The sialic acid was assayed using the method of Aminoff (1961).

Assay of serum sialic acid: Free serum sialic acid was assayed like that of erythrocyte surface sialic acid. However, the free serum sialic acid was assayed without consideration to prior mild hydrolysis.

Assay of Sialidase: The assay of sialidase was carried out using the thiobarbituric acid assay method (Warren, 1959; Aminoff, 1961).

Statistical analysis: All the values obtained from the individual animal parameters in both the infected and control groups were pooled together as pre-and post-infected values and expressed as mean±SD. The mean values for the various parameters obtained from the control Savannah Brown bucks were compared with those of the infected group using Student's t-test (Winer, 1971).

RESULTS

Parasitaemia: All goats belonging to the infected group developed parasitaemia. The prepatent period varied between 7 and 11 days. After establishing a patent infection the mean parasitaemia continued to rise and reached a mean peak of 5×10^4 (i.e., +++) per mL of infected blood 27 days post infection. The parasitaemia then decreased up to day 51 when no parasites were detected. The parasitaemia rose again, fluctuating, throughout the period of investigation (Fig. 1). The control goats remained aparasitaemic throughout the course of the investigation.

Serum sialidase activity: The mean sialidase activity in the infected goats was increased minimally, while such changes did not occur in the control goats. Statistically significant differences occurred between the mean

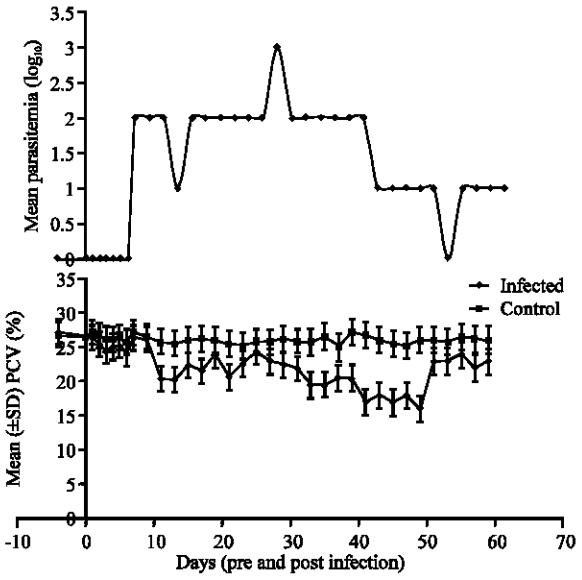

Fig. 1: Mean (±SD) PCV in relation to parasitaemia during *Trypanosoma evansi* infection in savannah brown bucks

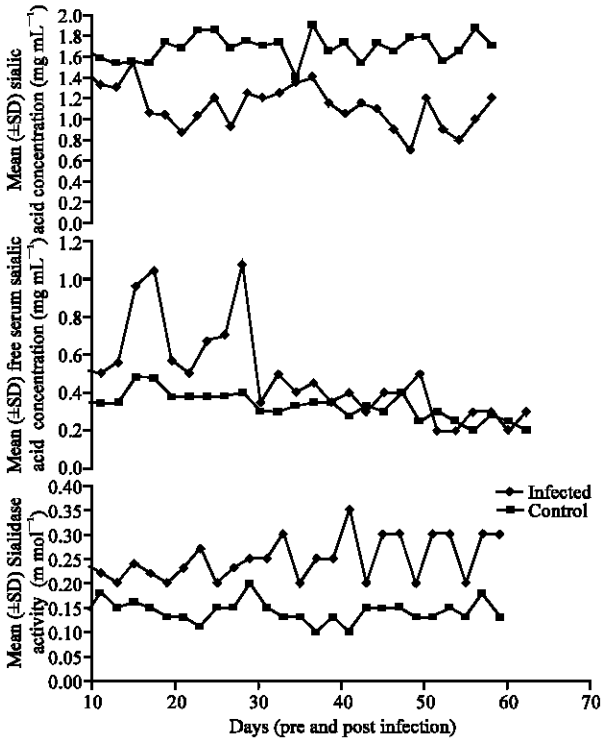

Fig. 2: Sialidase activity in relation to free serum and erythrocyte surface sialic acid concentrations in *T. evansi* infected and control Savannah Brown bucks

sialidase activity in the infected goats and that in the control goats on days 21, 23, 27, 33 and 37 post-infection (Fig. 2).

Erythrocyte surface sialic acid: A gradual decline in the mean erythrocyte cell surface sialic acid concentration was observed starting on day 5 post-infection. However, significant drops in the mean surface sialic acid occurred on days 21 (0.87+0.31 mg mL^{-1} and 27 (0.93+0.12 mg mL^{-1}). The mean concentrations in the control goats were 1.83+0.58 mg mL^{-1} on day 0, 1.68+0.53 mg mL^{-1} on day 21 and 1.68+0.22 mg mL^{-1} on day 27 post-infection. Statistically significant differences ($p<0.05$) in the mean erythrocyte surface sialic acid concentrations occurred between the infected and control goats on day 5 and between days 17 to 27 (Fig. 2).

Serum sialic acid concentrations: Elevated levels of mean serum sialic acid were observed on days 15 (0.96+0.11 mg mL^{-1}) and 17 (1.04+ 0.4 mg mL^{-1}) post-infection. Similarly, an elevation occurred on day 27(1.07+0.06 mg mL^{-1}) post-infection (Fig. 2). The mean serum sialic acid concentrations in the control group were not elevated during the course of the study.

Statistically significant ($p<0.05$) difference between the mean serum sialic acid concentrations in infected goats and the control goats occurred on days 15, 17 and 27 post-infection.

Packed Cell Volume (PCV): The mean PCV value decreased from 26.60+0.89% on day 0 to as low as 19.50+2.12% on day 33 post-infection in the infected group. However, the mean PCV in the control (uninfected) goats remained relatively within the normal range 25.0+1.26 and 27.25+1.5. A statistically significant difference occurred in the mean PCV values between the infected and control groups on days 11, 13, 21, 33, 35 and 37 post-infection (Fig. 1)

Haemoglobin concentrations: The mean haemoglobin concentration declined in the infected group from 8.94+0.21 g dL^{-1} on day 0 to 6.50+0.70 g dL^{-1} on day 33 post-infection. The mean haemoglobin concentrations in the control goats remained relatively normal (9.15+0.44 g dL^{-1} on day 0 and 8.48+0.35 g dL^{-1} on day 33). Statistically significant difference ($p<0.05$) occurred between the infected and control group on days 11, 13, 21, 33, 35 and 37 post-infection.

DISCUSSION

The prepatent period in this study varied between 7 and 11 days as earlier reported by Verma and Gautam (1978) on *T. evansi* infected buffalos and cow calves, by Stephen (1986) on *T. evansi* infected goats. However, it was longer than reported by Audu *et al.* (1999) on *T. evansi* infected Yankasa sheep. The intensity of the parasitaemia ranged between 1-plus (+) and 3-plus (+++) with the highest levels of parasitaemia recorded during the early phase of the infection. This pattern was also reported by Anosa and Isoun (1980) on *T. vivax* infected small ruminants.

The *T. evansi* infected goats developed moderate levels of anaemia as evident by gradual fall in PCV and haemoglobin concentrations. The observed decreases in PCV and haemoglobin concentrations coincided with the fluctuating parasitaemia, which suggest that living trypanosomes were responsible for the progressive development of anaemia, as previously observed by Ogbadoyi *et al.* (1999). It was also found in this study that there was a gradual increase in serum sialidase activity and the activity coincided in some cases with the decrease in erythrocyte surface sialic acid and increase in free serum sialic acid concentrations. This suggests that *T. evansi* produced sialidase, cleaving red cells sialic acid into plasma, further supporting the report that *T. evansi* infection decreased the sialic content of erythrocyte

membranes and increased sialic acid content in plasma (Walia *et al*., 1996). The observation in this study of the decrease in erythrocyte surface sialic acid, which coincided with the increase sialidase activities further support this. Furthermore, free serum sialic acid was found to have increased with the appearance of the trypanosomes with the highest value being recorded during the peak of parasitaemia and highest sialidase activities by *T. evansi*. Therefore the decline in erythrocyte surface sialic acids with increase in free serum sialic acid may be readily explained. However, the decline in erythrocyte surface sialic acid did not produce significant increase in free serum sialic acid in *T. vivax* infected cattle (Esievo *et al*., 1982). It was suggested that the sialic acid removed from the erythrocytes surface was metabolized or detoxified as soon as it was produced (Esievo *et al*., 1982). Indeed, Kraemer (1966) also suggested that the enzyme sialyl transferase, present in calf thyroid gland (Spiro and Spiro, 1968) regenerated sialic acid on the surface of the cells.

The findings of very significant increase in free serum sialic acid coinciding with significant decease in red cell surface sialic acid was contrary to the findings of Esievo *et al.* (1982) which appears to suggest low levels of or absence of sialyl tranferase in goats.

It has been shown that *T. vivax* produced neuraminidase enzyme both *in vitro* and *in vivo* (Esievo, 1979; Esievo *et al*., 1982) in *T. vivax* infected cattle and this enzyme have been reported to cleave-off erythrocyte surface sialic acid in cattle (Esievo *et al*., 1982). Therefore, the findings that a reduced erythrocyte surface sialic acid occurred at the same time with an increase number of trypanosomes in circulation accompanied by development of anaemia was an evidence that the trypanosomes might be producing neuraminidase which in turn cleaves off erythrocyte surface sialic acid thus rendering them more prone to phagocytosis as observed previously by Esievo *et al.* (1982) in *T. vivax* infected cattle. A similar pathophysiological mechanism may be operational in the development of anaemia in Savannah brown buck as a result of *T. evansi* infection.

CONCLUSIONS

It is reasonable to postulate from the present study that as *T. evansi* multiplies in the peripheral circulation of the infected goats, it produced neuraminidase which cleaved off erythrocyte surface sialic acid which caused damage to the cells and render them more prone to haemolysis by the reticuloendothelial system.

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