RELATIVE EFFICACY OF TWO TRYPANOCIDES ON THE TREATMENT OF TRYPANOSOME BRUCIE INFECTED GRASSCUTTER (*THRYONOMYS SWINDERIANUS*)

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ABSTRACT

*The effects of two trypanocides (Berenil®) and Pentezine ®) on Trypanosoma brucei infection of captive reared grasscutters (Thryonomys swinderianus) was investigated. Weight, serum proteins and enzyme changes were monitored during the study.Results obtained showed marked difference as the mean ±**SD for total protein,** aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP)increased after the infection but approached the original values post treatment in the grasscutters. However, the values for globulin followed an opposite pattern while moderate weight changes were observed. The trypanosome organism elicited severe parasitaemia and anaemia in the grasscutters after a prepatent period of 5 to 7 days. Curative treatment of the infected rodents using 0.35mg/kg (Berenil ®) and Pentezine ®) given on the 7th day of infection resulted in momentary signs of recovery of the animals.*

Keywords: Grasscutter, Trypanosoma brucei,Biochemical changes, Trypanocides

INTRODUCTION

The role of domestication in wildlife conservation extends beyond making animal proteins available and curbing illegal hunting (Anderson 1991). It affords wildlife practitioners the ability to carry out certain experiments in order to have a comprehensive view of what could possibly be happening in the wild and to have a better disease control in wildlife domestication or micro-livestock production practices (Ado 1997 and NRC 1991). Wildlife values can also be extrapolated to provide information to conservationists, which help in the eventual achievement of the entire goal of conservation.

Considering the increasing interest in grasscutter farming, it becomes very important to study the diseases they are susceptible to. The major diseases of grasscutters include pulmonary congestion, pneumonia, septic wounds, ruptured uterus, orchitis with septicemia, gastro-intestinal obstruction, and gastroenteritis (Merck manual 2010; Opara and Fagbemi 2010). The gastrointestinal obstruction and gastro enteritis may be caused by helminthes. Opara and Fagbemi (2008) also suggested that wild grasscutters could serve as efficient reservoir hosts for agents of African trypanosomosis. This in the long-run reduces the meat quality and the profitability of the domestication business on the part of the farmer.

Trypanosomosis has been reported to be a very important economic disease, which makes the rearing of livestock such as cattle, sheep, goats, pigs, horses, donkeys and dogs in the forest and some parts of the savanna hazardous (Oppong, 1999). Trypanosomosis and Streptothricosis were responsible for the closing down of a cattle fattening scheme in 1958 in the East Central State of Nigeria due to losses from these diseases (Jordan 1986).The absence of a vaccine and the ability of the parasites to develop drug resistance as well as the wide distribution of *Glossina* spp., the vectors and other mechanical arthropod transmitters of the disease make trypanosomosis a very formidable disease to deal with (Dräger&Mehlitz 1978; Mattoli*et al.*, 1990).

The study is however focused on the first group which is the African trypanosomosis group caused by salivarian trypanosomes. This condition is characterized by an acute fever,

anaemia, emaciation and mortality (Mattioli *et al.*,1990). In livestock and dogs, the condition is transmitted principally by Glossina, the tse-tse fly. In grasscutters, studies on the occurrence of trypanosomosis have been carried out and it has been ascertained that the plasma biochemical parameters of the grasscutters are altered as a result of infection with trypanosomosis (Opara and Fagbemi, 2008). Undulating temperature is a clinical feature in animals infected with trypanosomes. It has also been ascertained that leucopaenia could result from trypanosomiasis infection in grasscutters (Seifert 1996).

The probability of Trypanosome infection in grasscutters gives a great concern to both conservation efforts and intending farmers in regions that are tsetsefly infested. It is also necessary to examine the efficacy of trypanocides and further advice on the treatment of trypanosomosis as this is very important for public health reasons and effective grasscutter farming. This study examined the possibility of infection of grasscutters (*Thryonomys swinderianus*) with *Trypanosoma brucei*, its overall effect on its health status and consequent productivity in the animals. A further aim of the study was to examine the efficacy of two trypanocides in the infected grasscutters.

MATERIALS AND METHODS

Management of the animals

Twenty sexually mature 5 month old grasscutters comprising 10 males and 10 females weighing averagely 1.3kg were purchased from Cotonou for this study. Each of the grasscutters was fed with an average of 0.4kg of *Pennisetum purperum* and 0.1kg concentrate daily and water was provided *ad libitum*. The animals were randomly divided into groups A and B. All the animals were weighed using a 50-kg capacity top-loading weighing balance. They were allocated to the same pen under uniform atmospheric conditions in a well-ventilated house. They were regularly fed and allowed a 2-week acclimatization period. The composition of concentrate diet fed to the grasscutter is presented in table 1.

Table 1: Composition of concentrate diets fed to captive grasscutters

Ingredient	Inclusion level (g/100 g dry mass)
Palm kernel meal	18.25
Cassava root meal	10.00
Soybean meal	20.00
Maize	28.00
Wheat bran	22.00
Di-calcium phosphate	1.00
Common salt	0.50
[1]Vitamin/mineral premix	0.25

[1]Vitamin/mineral premix composition: vit A (800 IU), vit D (3000 IU), vit E (8 IU), vit K (2 mg), vit B_1 (1 mg), vit B_2 (2.5 mg), vit B_{12} (5 mg), Niacin(10 mg), Panthothenic acid (5 mg), Antioxidant (6 mg), Folic acid (0.5 mg), Choline (150 mg), Iron (20 mg), Manganese (80 mg), Zinc (50 mg), Cobalt(0.22 mg), Iodine (2 mg) and Selenium (0.1 mg).
(Modified from *Karikari, 2009)*

Handling andInfection

The animals were handled according to standard procedures with care taken so as not to injure

them. They were usually picked up from the cage by a handling net, kept in a carton for weighing with the weight of the carton subtracted from the weight of each animal and the carton. Each animal was screened for endoparasites like trypanosomes and other blood parasites using standard techniques.

The strain of trypanosome used was the virulent *Trypanosoma brucei* isolated from cattle and after passage into albino rats from the National Trypanosomosis Research Institute, Kaduna, Nigeria. 0.4ml of infected albino rat blood was injected into each of the animal intra-peritoneally.

Sample collection

The grasscutters were carefully restrained according to method described by Donovan (2008) for bleeding before and after infection and after treatment. Whole blood samples were collected in heparinised and non-heparinised sample bottles for hematology and serum analysis respectively.

Treatment

The infected animals were treated with two brands of Diminazene aceturate; pentezine®and berenil®, 8 days after infection when the parasitaemia was 10^6/ml of blood in each cane rat. The dosage for both treatments was 0.35mg/kg body live weight using the intramuscular route according to the manufacturer's specification.

Weight changes measurement

The grasscutters were placed in transportation cages with predetermined weight, which was then subtracted from the total weight to give the weight of each of the grasscutters. This was done at pre-infection, mid-infection and post-treatment stages of the study.

Sample analysis

Blood samples were analysed for serum total protein and liver enzymes. Serum total protein was determined by Biuret method; Albumin, *Alanine aminotransferase* (ALT), *aspartate aminotransferase* (AST), and *alkaline phosphatase* (ALP) were determined using standard commercial test kit (RANDOX Laboratories Ltd., Ardmore, Diamond Road, Crumlin Co. Auturm, UK) in accordance with the instructions of the manufacturer.

Statistical analysis

Results were expressed as the means ± standard deviation of the values for each parameter in each of the groups before infection, after infection and after treatment and these values were compared using ANOVA test.

RESULTS

From the results (Table 1), there was significant increase in live weight after treatment against experimental trypanosomosis in the animals using Berenil ®.

Significant changes ($p<0.05$)were observed for weight in both groups where there was a remarkable decrease post infection and increase ten days post treatment in group A.

Mean ± SD value before infection was 7.08± 0.41for total protein, 58.4 ± 10.92 for AST, 31 ± 1.58 for ALT and 102 ± 26.01 for ALP. However, when compared with similar set of values post infection, noticeable increase was observed as serum total protein was 7.6 ± 0.49 in group A and *aspartate aminotransferase* (AST), *alanine aminotransferase* (ALT),and *alkaline phosphatase* (ALP) were 68 ± 12.10, 31.5 ± 4.45 and 117.6 ± 4.51, respectively. The same trend was witnessed for these values in group B (Table 2) where serum total protein was 7.9 ± 0.39 and *aspartate aminotransferase* (AST), *alanine aminotransferase* (ALT), and *alkaline phosphatase* (ALP) were 73 ± 7.79, 33.5 ± 1.73 and 120.5 ± 9.11, respectively.

Furthermore, after treatment with the trypanocides, decrease in these set of values was evident with total protein being 7.5 ± 0.28 in group A and *aspartate aminotransferase* (AST), *alanine aminotransferase* (ALT), and *alkaline phosphatase* (ALP) were 63 ± 1.41, 31.5 ± 2.12 and 97 ± 5.66, respectively as against the previous values before treatment with Berenil®. However, full comparison for those in group B could not be made because of the high mortality recorded. Appreciable changes were observed for globulin value in group B as compared to group A but both had increase albumin levels post infection.

Gross changes observed included severe anaemia, pulmonary congestion and oedema, hepatic congestion, renal congestion and mild splenomegaly. There was no significant effect of the trypanocides on the *T. brucei* infected grasscutters.

Table I: Weight and serum biochemical values of cane rats infected with *T. brucei* and treated with Berenil®

Parameter	Pre infection	Post infection (7 days)	Post treatment (7 days)	Post treatment (10 days)
Weight (kg)	1.29±0.07	1.3±0.12	1.2±0.00	1.25±0.07
Total protein (g/dl)	7.08±0.41	7.6±0.49	7.5±0.28	6.65±0.21
Albumin (g/dl)	3.78±0.39	4.64±0.34	4.6±0.14	4.15±0.21
Globulin (g/dl)	3.3±0.34	2.96±0.44	2.9±0.42	2.5±0.42
A:G ratio	1.02±0.36	1.56±0.30	1.2±0.28	1.65±0.35
AST IU/l	58.4±10.92	68±12.10	63±1.41	70±25.46
ALT IU/l	31±1.58	31.6±4.45	31.5±2.12	35.5±7.78
ALP IU/l	102±26.01	117.6±4.51	97±5.66	117±16.97

Table 2: weight and serum biochemical values of cane rats infected with T. brucei and treated with Putezine®

Parameter	Pre infection	Post infection (7 days)	Post treatment (7days)
Weight (kg)	1.34±0.11	1.35±0.06	1.33
Total protein(g/dl)	7.38±0.26	7.9±0.39	7.5
Albumin (g/dl)	4.22±0.19	4.65±0.50	4.2
Globulin (g/dl)	3.02±0.25	3.5±0.36	3.3
A:G ratio	1.38±0.16	1.3±0.29	1.2
AST IU/l	66±7.78	73±7.79	57
ALT IU/l	27.6±4.22	33.5±1.73	29
ALP IU/l	110±12.33	120.5±9.11	127

DISCUSSION

Detection of the effect of the trypanosome infection could be done by deductions from the values of parameters such as of serum protein viz Total Proteins, Albumin and Globulin as well as the liver enzymes; *Alanine aminotransferase* ALT, *aspartate aminotransferase* AST, *alkaline phosphatase* ALP (Bush 1991, Washington1996). An increase in albumin level (hyperproteinaemia) indicates dehydration, and thus provides information about the function of the liver, kidneys and digestive system. Globulin levels reflect underlying inflammation and/or antibody production. Increased levels of globulins are often associated with infectious diseases, immune-mediated disease, and some

types of cancer. The enzymes ALT, AST and ALP are useful to diagnose hepatic dysfunction. The first two enzymes are often increased when there is hepatic inflammation, injury, or necrosis. The last one tends to increase when bile flow in the liver is decreased or in cholestasis (Doumas *et al.*, 1971).

The results show a mild but continuous increase in ALT, AST, and ALP following infection (7 days post infection) in the infected grasscutters, confirming the presence and activity of the infection. While ALT continued with marginal increase by post-infection, there was decrease in AST and ALP levels midway post infection. In fact, at this point the blood cells of the grasscutter were noticed to be crenated (an abnormality in red cells with unusual serrated edges) when viewed under the microscope together with recorded high parasitaemia. The relative increases in these liver enzymes may be attributed to anoxic/hypoxic injury to the hepatocyte following development of anaemia in the infected animals. The increase in the ALP may be due to mild hepatic cholestasis caused by swollen degenerating hepatocytes commonly observed in hepatic lipidosis (fatty degeneration) that accompanies hepatic tissue hypoxia in anaemic conditions. The Pre-patent period of infection with *Trypanosoma brucei* then can be agreed to be 5-7days in the infected grasscutters in this study. From the indication of the total protein value there is a mild dehydration.

The subsequent earlier decrease in AST compared to sustained mild increase in AST post-infection could be due to the relatively shorter half life of AST than ALT in which case AST is more rapidly cleared from serum. The increase indicated in the globulin concentration however implies the production of antibody and inflammation corroborating the presence of the infection. *Alanine aminotransferase* (ALT), *aspartate aminotransferase* (AST), and *alkaline phosphatase* (ALP) levels reflect the pathological effect of the blood protozoan on the liver, kidney and the digestive system of the grasscutters. The last enzyme i.e. *alkaline phosphatase* (ALP) tends to increase when bile flow in the liver is decreased thereby lowering the process of emulsification of fat, which further makes energy unavailable for the animal.

Although the animals were treated with two different brands of diminazene aceturate in all the infected grasscutters, they did not recover fully as there was an eventual 100 percent mortality.

Due to limited information on the literature on the subject matter, comparison with past reports is difficult. Nevertheless, this study serves to provide basal parameters in that aspect. However, Opara *et al*, 2006 reported total protein of male grasscutters as 7.0±10.29 and 7.4±0.37 for female; this is quite comparable to the values of 7.08±0.41 and 7.38±0.26 for male and female, respectively obtained from this study. The observed gross changes of marked anemia, pulmonary congestion and oedema, hepatic congestion, renal congestion and mild to moderate splenomegaly still characterize the pathology of African animal trypanosomosis (Anosa 1988).

The therapeutic effect of the trypanocides was not significant across group. Though there was an initial recovery, this was transient. This could beas a result of preparations used, originally meant for domesticated livestock. However, group A treated Berenil® showed apparent signs of recuperation.

CONCLUSION

Grasscutter is very susceptible to *Trypanosoma brucei* infection and this is note-worthy for possible effective preventive measures against natural infection and treatment especially when considered in the light of possible effect of domesticated stocks and its overall effect on conservation as well as public health. Further studies are needed to fully elucidate the effect of naturalTrypanosomosis in grasscutters as well as the general diseases affecting these animals.

The diagnostic approach employed in this work revealed the effect of the infection on the kidney and liver of the grasscutters before eventual death (high mortality recorded) as none of the trypanocides proved effective enough to effectively reverse the deleterious effects of the infection.

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