Therapeutic Effect of Berenil^{R} in Experimental Murine Trypanosomiasis using Stocks Isolated from Apparently Healthy Captive - Reared Grasscutters (*Thryonomys swinderianus*).

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ABSTRACT: We investigated the effects of a trypanocide (Berenil ^{R}) on Swiss albino mice infected with *Trypanosoma congolense* and *Trypanosoma vivax* stocks isolated from apparently healthy captive - reared grasscutters. The two trypanosome organisms elicited severe parasitaemia and anaemia in the mice after a pre-patent period of 6 to 10 days and 4 to 7 days for *T. congolense* and *T. vivax* respectively. *T. vivax* produced a more severe anaemia than *T. congolense.* At 10 days post infection, the red blood cell (RBC) indices, packed cell volume (PCV) and hemoglobin concentration (Hb) were significantly ($p< 0.01$) lower in mice infected with *T. vivax* than those infected with *T. congolense.* Curative treatment of the infected rodents using 1mg / kg Diminazene aceturate (Berenil ^{R}) given on the 10th day of infection resulted in complete recovery of the animals from the parasitaemia and anaemia. It appears that the grasscutter is trypano- tolerant and this is note- worthy for possible vaccine development in the future. [Nature and Science 2010;8(9):106-110]. (ISSN: 1545-0740).

Key words: Berenil, Murine, Trypanosomiasis, Apparently, Healthy, Wild, Grasscutters.

INTRODUCTION

Globally, wildlife has great potentials for meat production and serves as an important source of the highly desired animal protein to the people of Africa, both in urban areas and rural communities (Ajayi, 1975; Fonweban and Njwe, 1990). The preference for bush meat or the meat of commercially available game animals is widely accepted (Ajayi, 1975; Baptist and Mensah 1986; Fonweban and Njwe, 1990). However, with ever increasing human population and obvious protein shortage in Africa, there is the need for an exploration of other means to provide readily acceptable meat on short term basis.

Wildlife domestication has been recognized as a way of achieving this objective (Ajayi.1975).A few number of small mammal and crop farmers, trade or breed wild rodents (Fonweban and Njwe, 1990; National Research Council, 1991), but research studies in their domestication are producing conflicting results (Baptist and Mensah, 1986; National Research Council, 1991).

Among the wild rodents, the grasscutter, or cane rat or cane cutter is the most preferred (Asibey and Eyeson, 1973; Clottey 1981). Grasscutter (*Thryonomys swinderianus*) is a wild hystricomorphic rodent widely distributed in the African sub-region and exploited in most areas as a source of animal protein (Vos, 1978; Asibey, 1974; National Research Council, 1991). Being the most preferred (Martin, 1985) and most expensive meat in West Africa including Nigeria, Togo, Benin, Ghana and Cote d' voire (Baptist and Mensah, 1986; Asibey and Addo, 2000), it contributes to both local and export earning of most West African Countries (Asibey, 1969; National Research Council 1991; Baptist and Mensah, 1986; GEPC 1995; Ntiamoa-Baidu, 1998) and is therefore hunted aggressively. Unfortunately its collection from the wild is attended by destruction of the environment through the setting of bush fires by hunters (National Research Council, 1991; Yeboah and Adamu, 1995; Ntiamoa-Baidu, 1998). To alleviate this problem, attempts are being made in the sub-region to domesticate the grasscutter (National Research Council, 1991; Addo. 2002) and make it more readily available, gain economic benefit and also reduce the environmental destruction that accompanies its collection from the wild. For example, a major research programme on grasscutter has been initiated in Benin Republic under the project Benino-Allemand d' Aulacodiculture (PBAA) to select genetically improved grasscutter stocks adapted to life in captivity and to promote the rearing of the animal in rural and sub-urban environments (Baptist and Mensah, 1986).

Social-economical and zootechnical characteristics of raising grasscutter have been reported (Baptist and Mensah, 1986; Mensah et al, 1986; Holzer et al, 1986; National research Council, 1991; Awa-Ndukum et al, 2001), but there is little information on their disease status. However, preliminary studies on the captive grasscutter (Awah-Ndukum et al, 2001) in Cameroon showed the occurrence of ectoparasites such as Fleas (*Xenopsylla sp*) and endoparasites like Cestode (*Hymenolopsis sp*) and Nematode (*Heterakis sp*) in this animal. In another work by Yeboah and Simpson (2004) in Ghana, four species of ticks namely *Rhipicephalus simpsoni, Ixodes aulacodi, Ixodes sp* and *Haemaphysalis parmata* were the ectoparasites found while six species of helminthes parasites comprising 2 species of Cestodes (*Furhmanella transvalensis, Railettina mahone*) and 4 species of Nematode (*Longistriata spira, Trachypharynx natalensis, Paralibyostrongylus vondwei and Trichuris paravispicularis*) were equally found.

Reports by Opara and Fagbemi (2008), showed that grasscutters can be infected with trypanosomes, although without obvious clinical disease. The trypanosomes isolated were of the species pathogenic to ruminant livestock, such as *Trypanosoma congolense* and *T. vivax* (Soulsby, 1989). There is no report of the pathogenicity of isolated trypanosome stock on other species of animals. This work will therefore investigate the effects of *Trypanosom*a stock isolated from grasscutters on the physiological condition of Swiss Albino mice.

MATERIALS AND METHODS.

Laboratory animals used for this study were 20 Albino Swiss Mice, weighing between 20g – 25g purchased from Michael Okpara University of Agriculture Umudike, Abia state. They were divided into 2 groups of 10 mice, fed with commercial growers' mash (Zion feeds Nig Ltd) and drinking water provided *ad libitum.*

The trypanosomes used for the infection of the mice were *Trypanosoma congolense* and *T. vivax* stocks obtained from grasscutters with single infection of these parasites.

Before the commencement of the experiments, blood samples were taken from the mice by snipping their tails for presence of any parasite and estimation of their haematological values. Haemoglobin (Hb) concentration was determined by cynomethaemoglobin method, Packed cell volume (PCV) by capillary tube method, total Red blood cell (RBC) by the haemocytometer method and total White blood cell count (WBC) was determined by Giemsa stained slides method (Coles,1986).

Heparinized 75mm capillary haematocrit tube was filled with blood up to 2/3 of its length; while the other end was sealed with plasticine .It was centrifuged for 5 minutes at

1,200g and thereafter cut with a diamond pen above the buffy coat column. A single drop of plasma was read on the platform of the refractometer.

Each mouse was weighed before and after the treatments on Mettler PM 600 weighing balance.

A quantity of 0.5ml of blood containing each of the trypanosome parasites was administered intra-peritoneally into each of the mice in the different groups. This is such that mice in treatment group A (TA), each received 0.5ml of blood containing *Trypanosoma congolense* and those in group B (TB) each received 0.5ml of blood containing *T.vivax*. Parasitaemia was monitored daily until the parasites were observed in the blood of the mice, using the dark ground/ phase contrast buffy coat method (Paris et al, 1982).

After 10 days that the parasites were seen in the blood of the animals, samples were taken by snipping of the tails to evaluate the haematological values. The blood samples were collected 10 days post infection (10dpi) to allow for maximum harvest of the parasites (Murray et al, 1984).

Five mice, each from of the 2 groups were administered 1.0mg/kg body weight of Berenil. The remaining 5 in each group served as control. After 70 days (i.e. 80 days post infection, 80dpi), blood samples were again collected to determine the haematological parameters.

Results were expressed as the means ±standard errors of the means. Significant differences between means were determined using the students t test (Bailey, 1992).

RESULTS.

The results of the effects of Berenil administration in infected mice show that trypanosome organisms infecting the grasscutters are equally pathogenic to other animals.

A pre-infection packed cell volume (PCV) of 52% was obtained for the group of mice to be infected with *T. congolense*. This value is slightly higher than the normal value for healthy mice (Canadian Council and Animal Care (CCAC). This value decreased to 42% at 10dpi ($P<0.05$). By 21 days post infection (21dpi) following Berenil administration, PCV value increased to 44.45 (Table 1). In the case of mice infected with *T. vivax*, a pre-infection PCV value of 57.6% was obtained. This value decreased to 47% at 10dpi and was maintained at 21dpi, following Berenil treatment (Table 2).

The RBC values of 8.95millions/µl and 8.10 millions/µl blood cells were recorded at pre-infection with *T. congolense* and *T. vivax* respectively as shown in tables 44 and 45.These values are within the normal

range of 7.00 -13.00 millions/µl of blood cells for mice (Aiello and Mays,1999). There was a significant reduction in RBC (Tables 1 and 2) at 10dpi for both groups of mice infected with *T. congolense* and *T. vivax,* thus recording RBC values of 7.31 millions/µl and 6.33 millions/µl respectively. Following treatment with Berenil, the RBC values increased at 21dpi to 8.80 millions/µl and 8.21 millions/µl of blood cells respectively.

The pre-infection Haemoglobin concentrations (Hb) were 14.80g/dL and 15.62g/dL (Tables 1 and 2). These values are higher than the 10g/dL - 14g/dL range recommended by CCAC. These values later decreased to 14.12g/dL and 14.14g/dL at 10dpi.Following Berenil treatment, the haemoglobin concentrations returned to normal at 21dpi,giving 15.04g/dL and 15.76g/dL respectively (Tables 1 and 2).

White blood cell (WBC) counts obtained before infection of the mice with the two specimens of trypanosome were 15.77 X 10^3µL and 14.86 X10^3µL. These values are higher than the recommended normal range of 13.6 x10^3µL by CCAC for healthy mice. The values decreased to 11.09 x 10^3µL and 11.28 x 10^3µL at 10dpi (Tables 44 and 45). On treatment with Berenil, the values again increased to 14.56 X 10^3µL and 15.30 x 10^3µL at 21dpi.

The mean values for plasma protein (PP) were 6.54g/dL and 6.28 g/dL before infection of the mice with trypanosome stocks (Tables 1 and 2). These values decreased to 5.68 g/dL and 5.02 g/dL at 10dpi with *T. congolense* and *T. vivax* respectively. After the administration of Berenil, plasma protein values for the two of mice (TA and TB) increased to 6.25 g/dL and 6.20 g/dL at 21dpi.

Table 1: Effects of Berenil treatment on haematology and body weights of Albino Swiss mice infected with *Trypanosoma congolense* stock from grasscutters.

	PCV (%)	RBC (millions/µL)	Hb (g/dL)	WBC (X10^3µL)	PP(g/dL)	BW(g)
Pre-treatment	52.00±1.89	8.95±0.44	14.80±0.57	15.77±3.04	6.54±0.54	23.10±1.19
Control	49.60±2.06	7.80±0.55	16.38±0.88	17.82±2.91	7.68±2.91	24.80±0.90
10dpi	42.00^a±5.71	7.31±0.64	14.12^b±1.81	11.09±2.57	5.68^b±0.43	23.85±2.72
Control	48.00^b±2.71	7.30±0.32	15.50±1.10	14.08±2.52	6.62^a±0.65	25.04±1.54
21dpi	44.40^a±6.80	8.80±0.93	15.04^b±2.32	14.56^b±0.91	6.52^b±0.45	24.30^b±1.03

ab means on the same row with dissimilar superscripts are significantly different ($p<0.05$)

Table 2: Effect of Berenil treatment on haematology and body weights of Albino Swiss mice infected with *Trypanosoma vivax* stock from grasscutters.

	PCV (%)	RBC (millions/µL)	Hb (g/dL)	WBC (X10^3µL)	PP(g/dL)	BW(g)
Pre-treatment	57.60±5.96	8.10±0.88	15.62±1.84	14.86±2.30	6.28±0.48	23.66±1.60
Control	52.00±5.02	7.60±0.80	15.52±1.67	14.28±4.73	8.88±0.82	24.64±0.82
10dpi	47.00^a±7.02	6.33±0.46	14.14^b±2.44	11.28^b±4.08	5.02^b±4.68	23.37±0.67
Control	47.00^b±5.76	6.30^b±1.04	15.20±1.86	13.76±5.56	6.06±0.47	25.20±2.51
21dpi	47.00^b±6.38	8.20^b±0.47	15.76^b±2.14	15.30±2.28	6.20±0.52	24.38±0.62

ab means in the same row with dissimilar superscripts are significantly different ($p<0.05$

DISCUSSION.

This study evaluated the pathogenicity in mice of *Trypanosoma* organisms harboured by the grasscutters examined in Imo state.

It further determined the therapeutic effects of Berenil in experimental trypanosomiasis of the Albino Swiss mice. The disease was diagnosed based on the degree of Parasitaemia and anaemia as described by Murray and Black, (1985).

Trypanosoma congolense parasites were detected in the blood of the mice after a pre-patent period (PPP) of 7 – 10 days. This result is not in agreement with Kalu and Esuruoso,(1985) who obtained a PPP of 4 – 5 days in *T .congolense* infection of mice. This discrepancy in the results may not be unconnected with the wide variation in clones and strains infectivity of *T. congolense* for laboratory rodents as reported by Hoare,(1970).A pre-patent period of 8 – 10 days was observed in the mice challenged with *T. vivax* . This result is consistent with the findings of Seifert,(1996) who stated that trypanosomes appear in the blood of most animals exposed to infection with *T. vivax* after 8 – 10 days.

The significant fall in the PCV,RBC and Hb values in the mice after infection showed that the mice developed anaemia after they were challenged with *T. congolense* and *T. vivax.* This result is in agreement with pervious findings by Anosa,(1977); Gardiner et al,(1981); Grootenhuis et al,(1990) and Trail et al,(1992)

There were no significant changes in the values of plasma protein before, during and after infection

with either *T. congolense* or *T. vivax.* Similar results had been reported by Ikede, (1972).

Berenil at a dose of 1.0mg/kg body weight given subcutaneously at 10 days post infection (10dpi) with trypanosomes led to complete cure in the animals. All treated animals responded to the treatment and survived after 21 days. Similar findings in cattle, sheep and goats have been reported (Arowolo and Uche, 1988).

It has been established that even lower doses such as 0.125mg/kg body weight of a similar trypanocide (Samorin®), resulted in complete cure in *T. brucei* infection of mice (Arowolo and Uche, 1988).

All infected untreated animals used as control in TA and TB died before the end of the experiment. This confirms the pathogenic nature of the trypanosomes encountered in the grasscutters and also the efficacy of the trypanocide in chemotherapeutic control of trypanosomiasis due to *T. congolense* and *T. vivax.*

Although a complete cure was obtained, it does appear that *T. vivax* is more resistant to Berenil than *T. congolense*, because mice infected with this later parasite responded faster to the drug used. Again, *T. vivax* produced more severe infection in this study than *T. congolense.*It has been reported by Losos,(1986) that *T. vivax* appears to be more virulent in west Africa.

CONCLUSION.

Trypanosomes isolated from grasscutters were detected in the blood of mice after a prepatent period of 7 to 10 days. The mice developed anaemia as shown by the resultant erythropenia, decreased PCV and Hb values but no changes in the value of plasma protein. Subcutaneous administration of Berenil in trypanosome infected mice led to complete cure from 10 days post infection.

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