

EFFECT OF CYMELARSAN[®] AND BERENIL[®] ON CLINICO-PATHOLOGICAL CHANGES IN RED FRONTED GAZELLES (*Gazella rufifrons*) EXPERIMENTALLY INFECTED WITH *Trypanosoma brucei*

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SUMMARY

Clinico-pathological changes due to *Trypanosoma brucei* infection in red fronted gazelles subjected to the stress of captivity and the effects of melarsamine hydrochloride (Cymelarsan[®]) (groups A and B) or diminazene aceturate (Berenil[®]) (groups C and D) were studied. The prepatent period due to the experimental infection with *T. brucei* strain Mkar/84/NITR/6 was 7-8 days. Pyrexia due to elevated body temperature, elevated respiratory rate, loss of body weight, significant ($P < 0.05$) decline in erythrocyte indices with palour of mucous membranes were observed. The infection was associated with two successive peaks of parasitaemia in the infected and untreated control (group E) that died between days 48 and 52 post-infection. Treatment with Berenil[®] (3.5 mg/kg) cleared the parasites from circulation which was followed by a relapsed parasitaemia that resulted in the death of all the gazelles between days 46 and 52 post-infection. Treatment of the gazelles subcutaneously with single doses of Cymelarsan[®] (0.3 mg/kg or 0.6 mg/kg) and intramuscularly with Berenil[®] (7.0 mg/kg) body weight, significantly ($P < 0.05$) modulated all the effects of the infection before the end of the study. The infection generally produced significant ($P < 0.05$) lymphopenia, eosinopenia, neutropenia and consistent monocytosis, which were more marked in gazelles treated with Berenil[®] (3.5 mg/kg) body weight and the infected untreated control (group E). In conclusion, the results showed that trypanotolerance in gazelles may not be absolute when stressed and that resultant trypanosomiasis can be arrested by treatments with either Cymelarsan[®] at 0.3 mg/kg and 0.6 mg/kg or Berenil[®] at 7.0 mg/kg body weights.

KEY WORDS: Chemotherapy, *Trypanosoma brucei*, red fronted gazelles.

INTRODUCTION

The clinico-pathological effects of trypanosomiasis have been extensively studied and reported among domesticated animals (Soulsby, 1982; 1994) and among captive and free-living wild animals in most parts of the world (Marie, 1998; Reichard, 2002). Most of the captive and free-living wild animals in West Africa are known to frequently serve as reservoirs of trypanosomiasis for domestic stock (Soulsby, 1982; Brossy *et al.*, 1999).

Break-downs in trypanotolerance increases under captive conditions due to immunosuppressive effects of stress related corticosteroid output (Young, 1978; Boere *et al.*, 2005; Mbaya, 2007). Most wild animals living naturally in their undisturbed habitat self-medicate themselves with medicinal plants in their environment, a process

referred to by conservation biologist as "*Medicatus naturalis*" (Clayton and Wolfe, 1995). This remains so, until they are subjected to adverse conditions of captivity, draught or intercurrent infections without access to such plants when fulminating and sometimes-fatal trypanosomiasis manifests (Silva *et al.*, 1995; Marie, 1998; Parija and Bhattacharya, 2001; Reichard, 2002).

Recently, outbreaks of trypanosomiasis due to *T. brucei* infection in red fronted gazelles (*Gazella rufifrons*) primarily associated with stress in Maiduguri and Abuja zoos, respectively were observed and reported (Mbaya, 2007). In view of the relative importance of this semi-domesticated wild ungulate, particularly in the semi-arid region of Northeastern Nigeria where they are reared alongside sheep and goats and the devastating consequence of trypanosomiasis, it was thought necessary to simulate *T. brucei* infection in red

fronted gazelles experimentally with a view to understanding its clinico-pathological effects and how it can be managed effectively with trypanocides.

MATERIALS AND METHODS

Experimental animals

Thirty apparently healthy red fronted gazelles of both sexes, aged between two to three years and weighing between 20 to 25 kg, were obtained directly from the wild under authorization of the Ministry of Environment, Borno state, Nigeria. They were housed in concrete floored and fly-proof pens throughout the experiment. All the animals were routinely screened for blood, intestinal and external arthropod parasites according to standard criteria (Soulsby, 1994).

Those that harboured trypanosomes were treated with DL- α -difluoromethyl ornithine (Merrill and Dow, USA) at 400 mg/kg orally for four consecutive days. Those infected with helminthes were treated with Morantel (Pfizer, USA) at 400mg/kg orally for four consecutive days, while those harbouring blood rickettsial organisms were treated with oxytetracycline hydrochloride at 1ml/10 kg body weight. They were allowed 40 days acclimatization before the commencement of the experiment. The gazelles were fed on wheat bran supplemented with bean husks, guinea corn and chopped cucumber, while water was provided *ad libitum*. They were handled in accordance with International ethics on animal welfare for bio-research (Broom and Johnson, 1993).

Source of trypanosomes

Trypanosoma brucei brucei (Mkar/84/Nitr/6) used for the study, were obtained from the Nigerian Institute for Trypanosomosis Research (NITR), Kaduna, Nigeria. The organism was first isolated in 1984 from a fatal outbreak of porcine trypanosomosis in Mkar in Benue State, Nigeria (Agu and Bajeh, 1986). It was identified based on morphology and negative Blood Inhibition and Infectivity Test (BIIT) and stabilized by four passages in rats before storage in liquid nitrogen. The stabilates were passaged twice in rats and then transferred into Red Sokoto goats. Blood from the infected goats was diluted with phosphate-

buffered glucose saline (PBSG, pH 7.2). Each gazelle was inoculated via the jugular vein with 0.5ml of blood from the goat containing 1.5×10^6 trypanosomes.

Experimental design

The infected gazelles were randomly separated into six groups (A, B, C, D, E and F) of five gazelles each. Groups A and B were treated subcutaneously with melarsamine hydrochloride (Cymelarsan[®]) at a single standard dose rate 0.3 and 0.6 mg/kg body weights, respectively, while groups C and D were treated intramuscularly with melarsamine hydrochloride (Berenil[®]) at a standard dose rate of 3.5 and 7.0 mg/kg body weights, respectively. On the other hand, groups E and F served as infected and untreated, and uninfected controls, respectively. All treatments commenced at the onset of parasitaemia, by day 8 post-infection.

Monitoring of clinical and haematological parameters

Body temperature was measured in °C using a rectal thermometer, respiratory rate by counting the rise and fall of the flank per minute; body weight was measured using a sensitive mobile scale (Quik fit, England) while conditions of the mucous membranes and hair coat were monitored every other day till the end of the experiment (52 days) according to standard methods (Radostitis *et al.*, 1997). Packed cell volume (PCV), red blood cell count (RBC), haemoglobin (Hb) concentration and white blood cell count (WBC) were determined according to standard methods (Schalm *et al.*, 1995). Eosinophil count was performed as for total WBC, but with a diluting fluid made up of 5ml of 2% eosin, 5ml acetone and 90 ml distilled water (Coles, 1985). All estimations were repeated every other day till the end of the experiment.

Diagnosis of infection

To establish parasitaemia, blood samples obtained from the jugular vein of the infected gazelles was examined using wet mounts and microhaematocrit buffy-coat method (Murray *et al.*, 1983), while the degree of parasitaemia was estimated every two days by the 'rapid matching technique' (Herbert and Lumsden, 1976).

Statistical analysis

Data obtained during the study were analyzed using two-way analysis of variance (ANOVA) to detect variations between groups at 95% confidence limit (Maed and Curnow, 1983).

RESULTS

All infected gazelles showed varying degrees of elevated body temperatures (39.8 ± 3.2 to 40.5 ± 3.2 °C) and respiration (27.8 ± 2.64 to 30.6 ± 2.76 breadth/minute) following the appearance of parasitaemia by day 8 post-infection (Figs. 1 and 3). Treatment with Cymelarsan® at 0.3 mg/kg and 0.6 mg/kg or Berenil® at 7.0 mg/kg body weights commenced at the onset of parasitaemia by day 8 post-infection. These treatment regimes effectively modulated the changes with the attainment of pre-infection values before day 52 post-infection.

Those treated with Berenil® at 3.5 mg/kg body weight or the infected and untreated control however, experienced unabated increase in body temperature and respiratory rates with the death of the gazelles occurring between days 46 - 52 post infection (Table 1).

All the infected gazelles showed varying degrees of weight loss following infection (Fig. 2). Thereafter, gazelles treated with either Cymelarsan® at 0.3 mg/kg and 0.6 mg/kg or Berenil® at 7.0 mg/kg body weights, respectively began to gain weight post-treatment. On the contrary those treated with Berenil® at 3.5 mg/kg body weight or the infected and untreated control experienced a significant ($P < 0.05$) weight loss of 3.4 ± 0.92 kg and 5.4 ± 1.16 kg from their pre-infection live weights respectively as compared to a gain of 5.8 ± 1.20 kg of the healthy controls.

TABLE 1: Prepatent period and mortality pattern of red fronted gazelles experimentally infected with *T. brucei* and treated with either Cymelarsan® or Berenil® and their controls

Groups	Treatments	No. of animals	Prepatent periods. Mean \pm S.D	Mortality No. (%)	Time of death: Days post infection.
A	Cymelarsan® 0.3 mg/kg body weight	5	7.2 ± 0.34	0	-
B	Cymelarsan® 0.6 mg/kg body weight	5	7.8 ± 0.35	0	-
C	Berenil® 3.5 mg/kg body weight	5	7.8 ± 0.35	5(100)	46 ¹ , 52 ⁴
D	Berenil® 7.0 mg/kg body weight	5	7.2 ± 0.34	0	-
E	Infected/untreated control	5	8.8 ± 0.35	5(100)	48 ¹ , 52 ⁴
F	Uninfected control	5	0	0	-

^{1,4} Superscript indicate number of gazelles that died on that day post infection

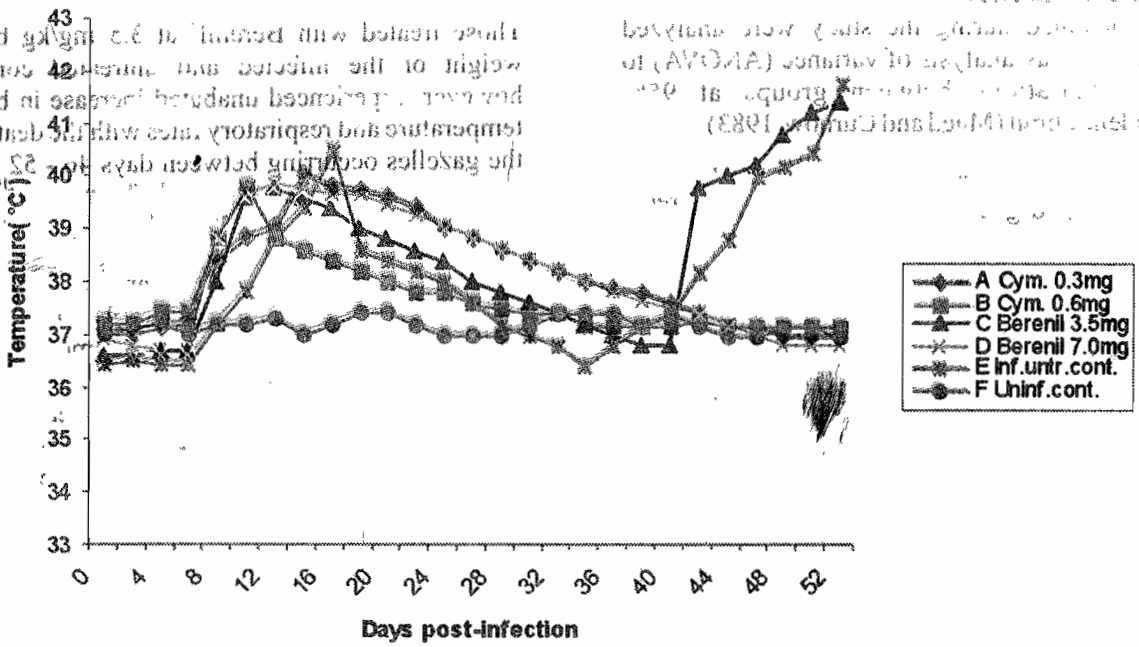


Fig. 1: Mean temperature (°C) changes of red fronted gazelles infected with *T. brucei* and treated with either Cymelarsan® or Berenil® and their controls

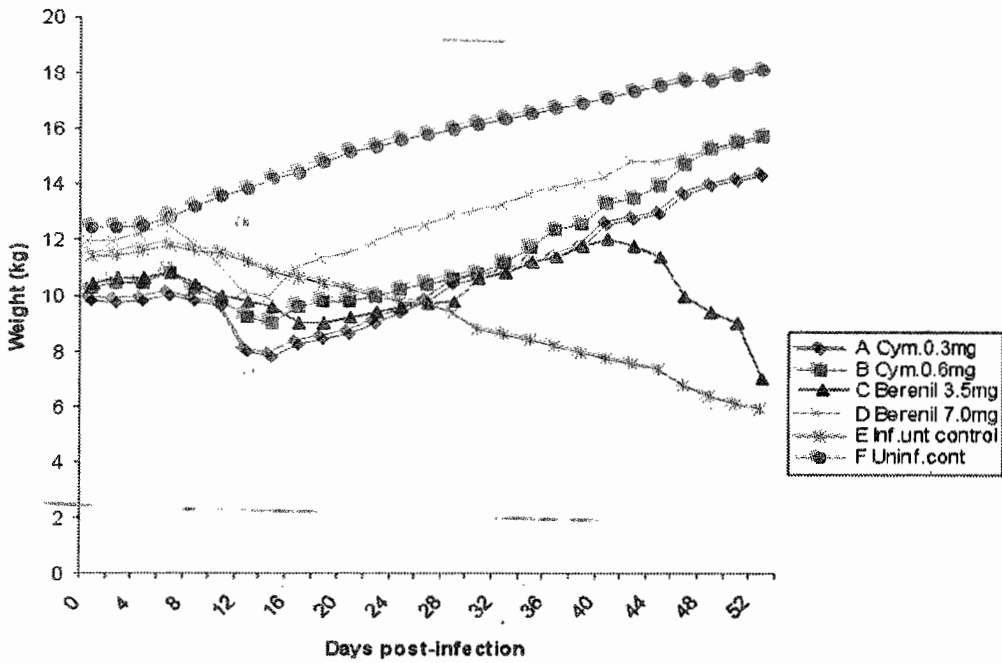


Fig. 2: Mean weight (kg) changes of red fronted gazelles infected with *T. brucei* and treated with either Cymelarsan® or Berenil® and their controls

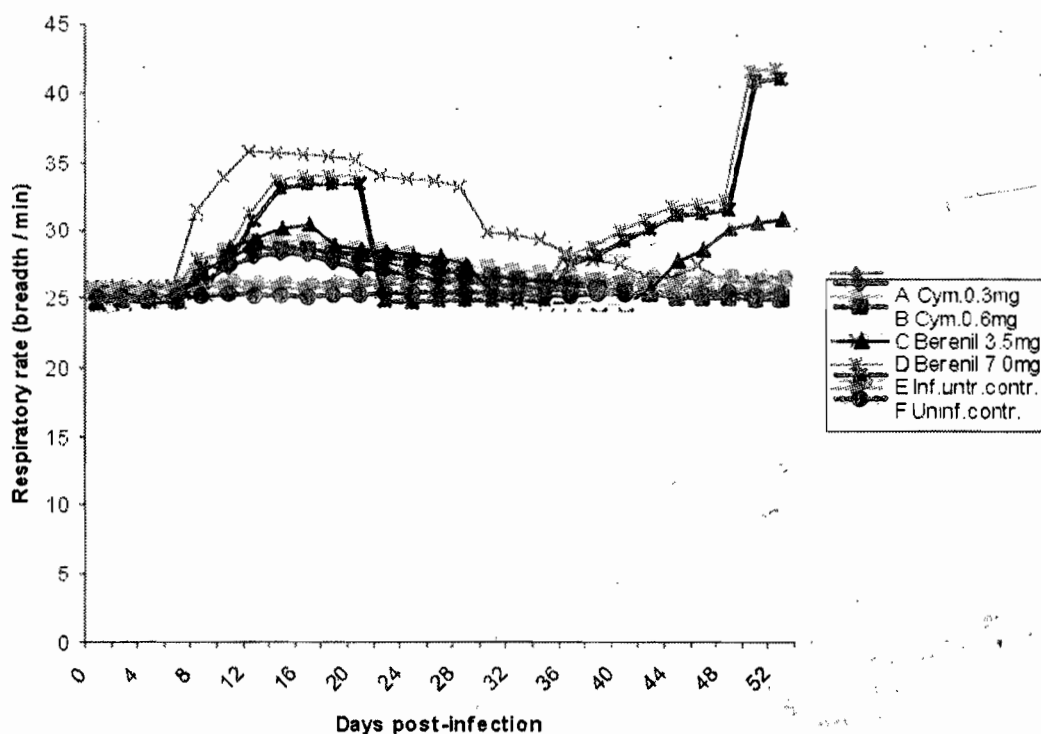
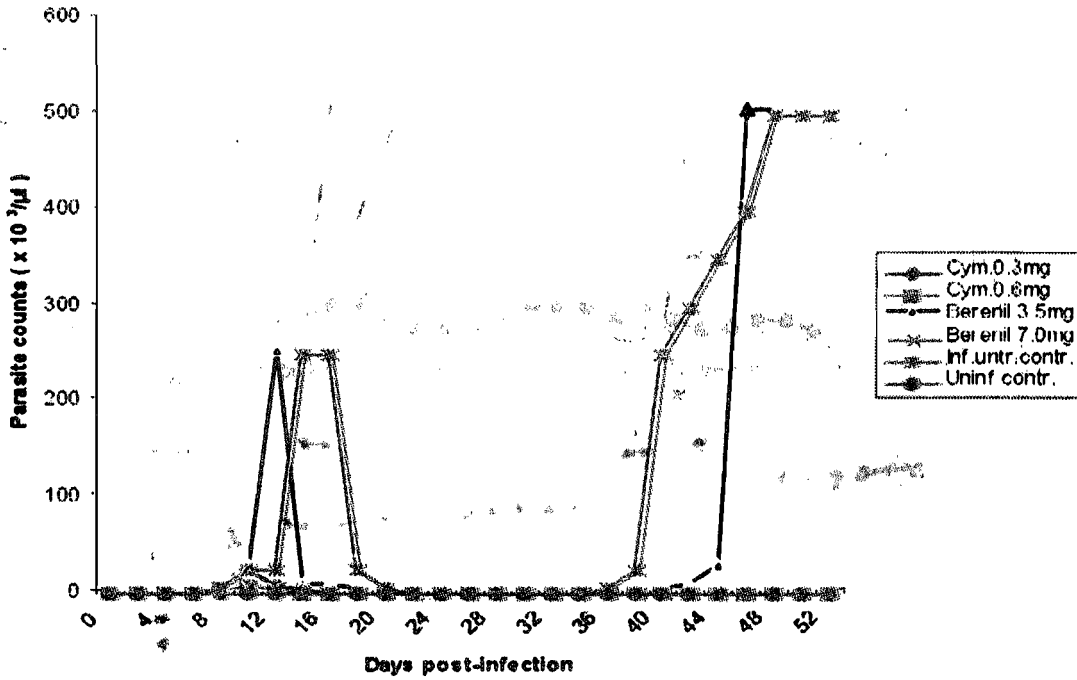


Fig. 3: Mean respiratory changes (breath / min.) of red fronted gazelles infected with *T. brucei* and treated with either Cymelarsan® or Berenil® and their controls. Key: Day of treatment (arrowed).

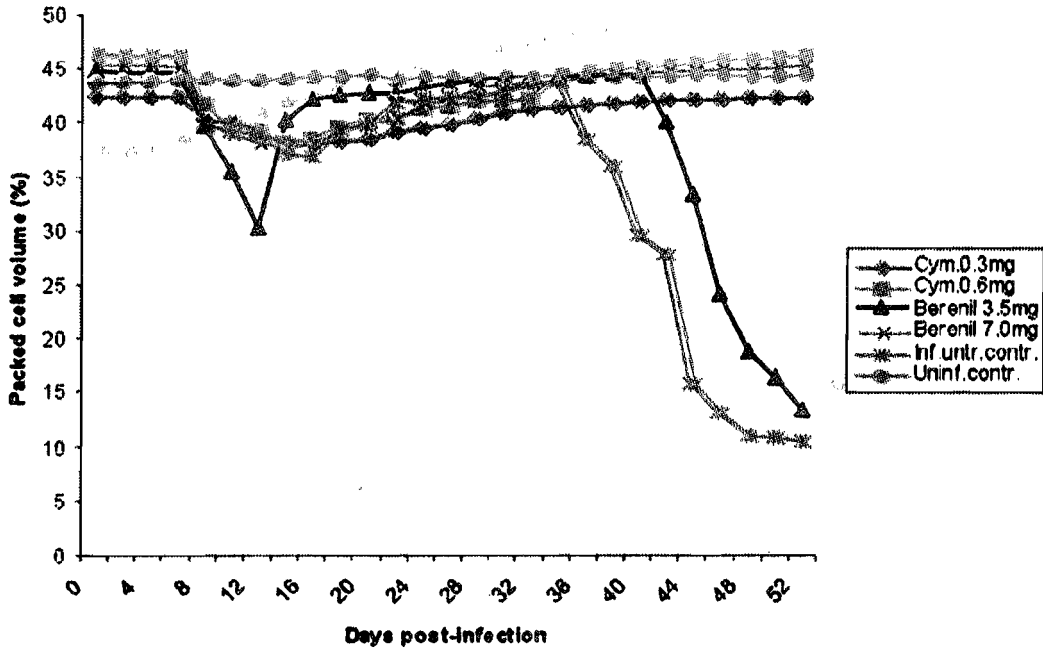
Trypanosomes were first detected in circulation with a mean parasite count of $5.5 \times 10^3 / \mu\text{l}$ for all infected groups following infection with a pre-patent period of 7 - 8 days. Following treatment with Cymelarsan® 0.3 mg/kg and 0.6 mg/kg body weights or Berenil® at 7.0 mg/kg body weight no relapse parasitaemia or deaths were encountered. However, gazelles treated with Berenil® at 3.5 mg/kg body weight had a count as high as $250.0 \times 10^3 / \mu\text{l}$ during the first wave and $500.0 \times 10^3 / \mu\text{l}$ during a relapsed parasitaemia. Successive peaks with similar values were equally encountered in the infected and untreated control (Fig. 4).

There was a gradual decline in the mean PCV, RBC and Hb values following infection in all infected groups (Figs. 5, 6 and 7). Thereafter, the values began to appreciate significantly ($P < 0.05$) with the attainment of pre-infection values before day 52 post-infection for all gazelles in groups treated with Cymelarsan® at 0.3 mg/kg and 0.6 mg/kg body weight or Berenil® at 7.0 mg/kg body weight, while those treated with Berenil® at 3.5 mg/kg body weight and infected and untreated control on the other hand, experienced a significant decline ($P < 0.05$) in PCV, RBC and Hb values, which remained unabated till values as low as $13.3 \% \pm 1.82$, $11.2 \times 10^6 / \text{mm}^3 \pm 1.67$ and $3.2 \text{ g/dl} \pm 0.9$ were attained, respectively.



Key: Day of treatment (arrowed)

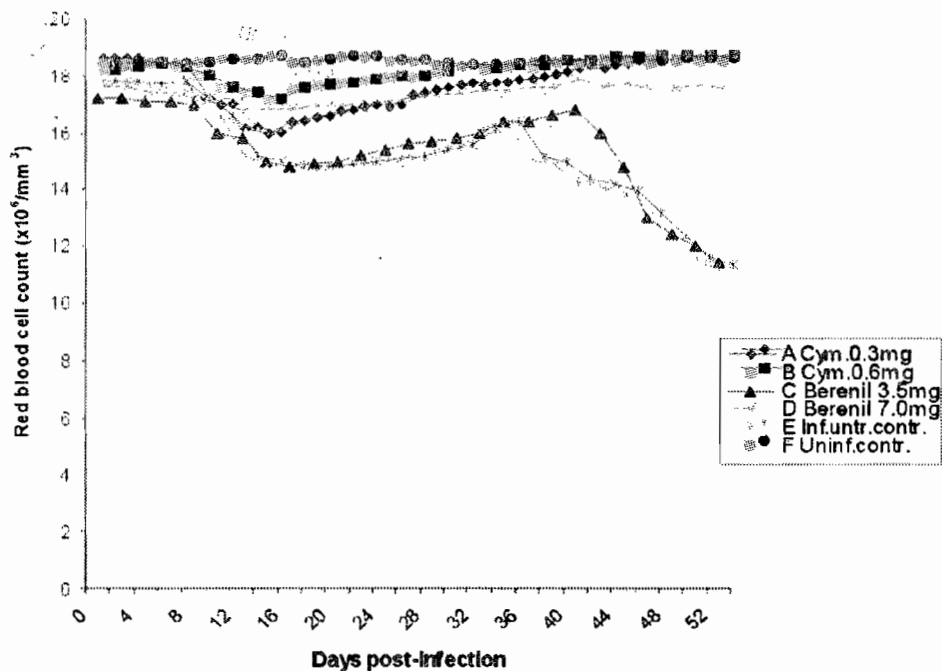
Fig. 4: Parasite counts (x 10³/μl) of red fronted gazelles infected with *T. brucei* and treated with either Cymelarsan[®] or Berenil[®] and their controls



Key: Day of treatment (arrowed)

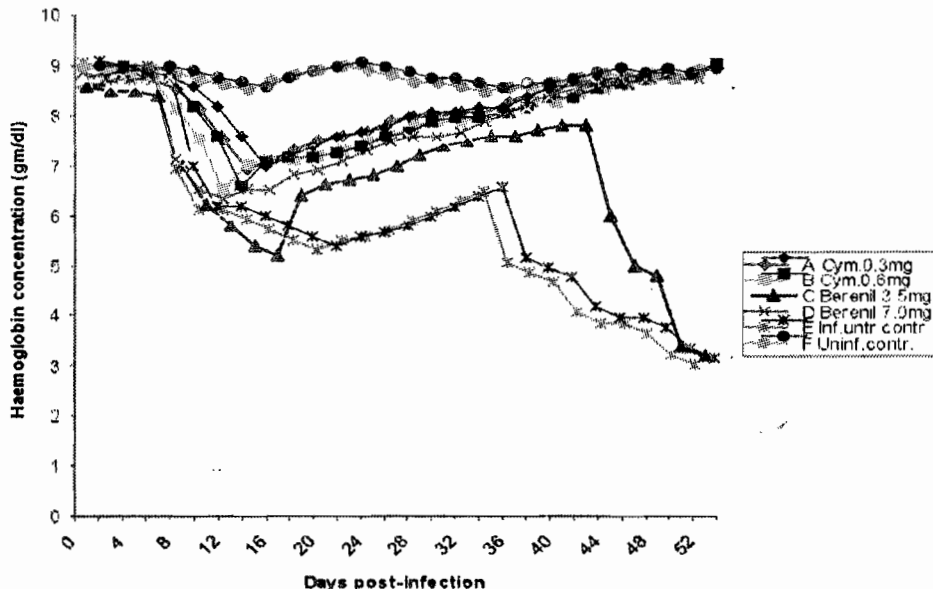
Fig. 5: Mean packed cell volume (%) in red fronted gazelles infected with *T. brucei* and treated with either Cymelarsan[®] or Berenil[®] and their controls

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Key: Day of treatment (arrowed)

Fig. 6: Mean red blood cell count ($\times 10^6/\text{mm}^3$) of red fronted gazelles infected with *T. brucei* and treated with either Cymelarsan® or Berenil® and their controls

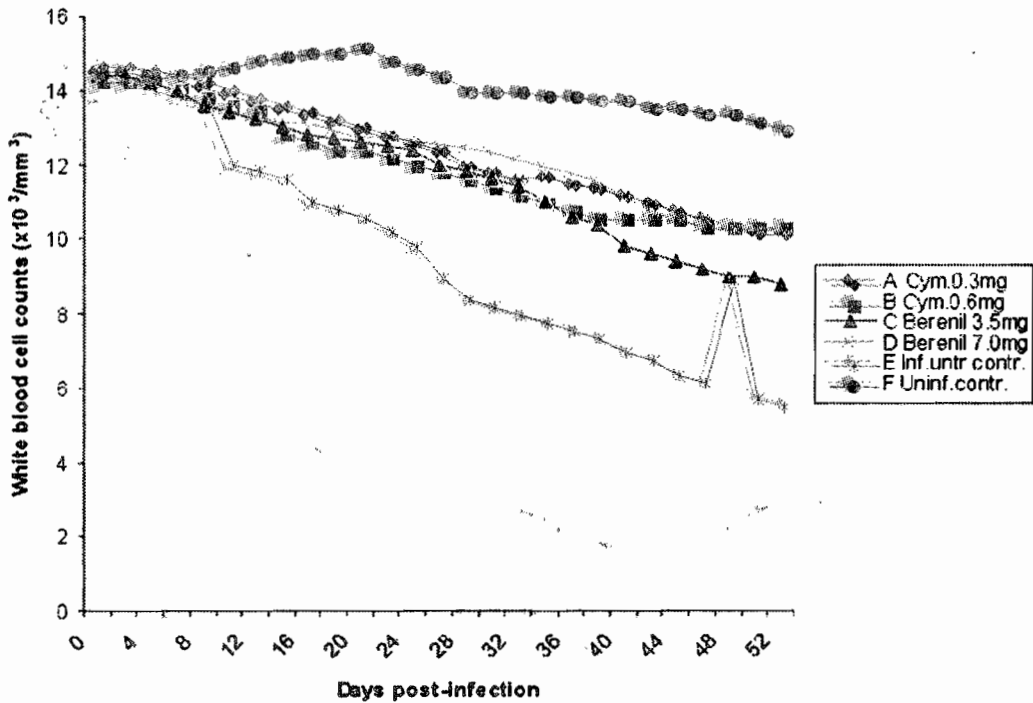


Key: Day of treatment (arrowed)

Fig. 7: Mean haemoglobin concentration (gm/dl) of red fronted gazelles infected with *T. brucei* and treated with either Cymelarsan® or Berenil® and their controls

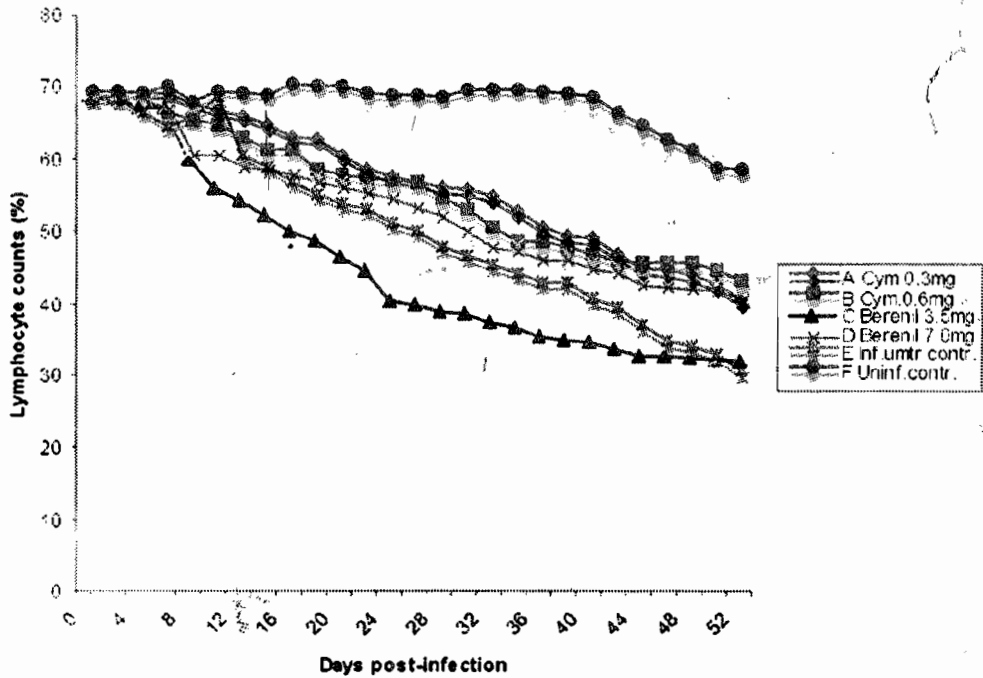
All infected groups experienced leucopenia and lymphocytopenia. The decline however was statistically more significant ($P < 0.05$) for groups C and E in relation to the other treatment groups (Fig. 8 and 9). On the other hand, a consistent monocytosis was experienced in all infected groups which were effectively modulated to pre-infection values for those treated with Cymelarsan® at 0.3mg/kg and 0.6mg/kg or Berenil® at 7.0 mg/kg. Exceptions were the groups

treated with Berenil® at 3.5 mg/kg and the infected and untreated control which experienced elevated values of $20.0 \% \pm 2.42$ and $45.0 \% \pm 3.35$, respectively (Fig. 10). Also following infection, neutrophil numbers continued to decline significantly ($P < 0.05$) for all infected groups (Fig. 11). Eosinophil counts, however, fluctuated slightly but remained within normal range (Fig. 12).



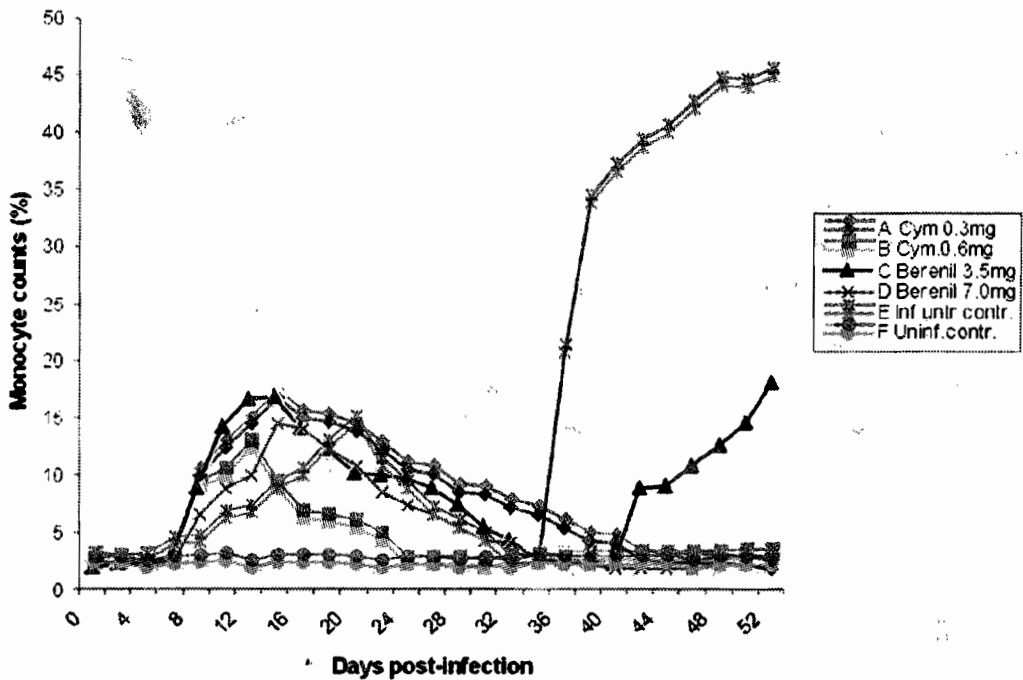
Key: Day of treatment (arrowed)

Fig. 8: Mean white blood cell counts ($\times 10^3/\text{mm}^3$) of red fronted gazelles infected with *T. brucei* and treated with either Cymelarsan® or Berenil®



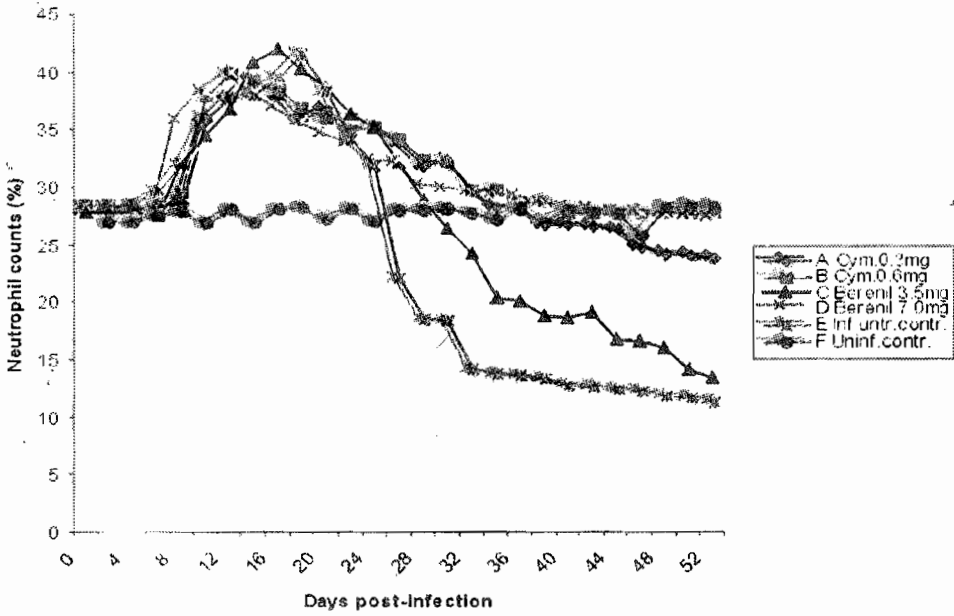
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Fig. 9: Mean lymphocyte counts (%) of red fronted gazelles infected with *T. brucei* and treated with either Cymelarsan® or Berenil® and their controls



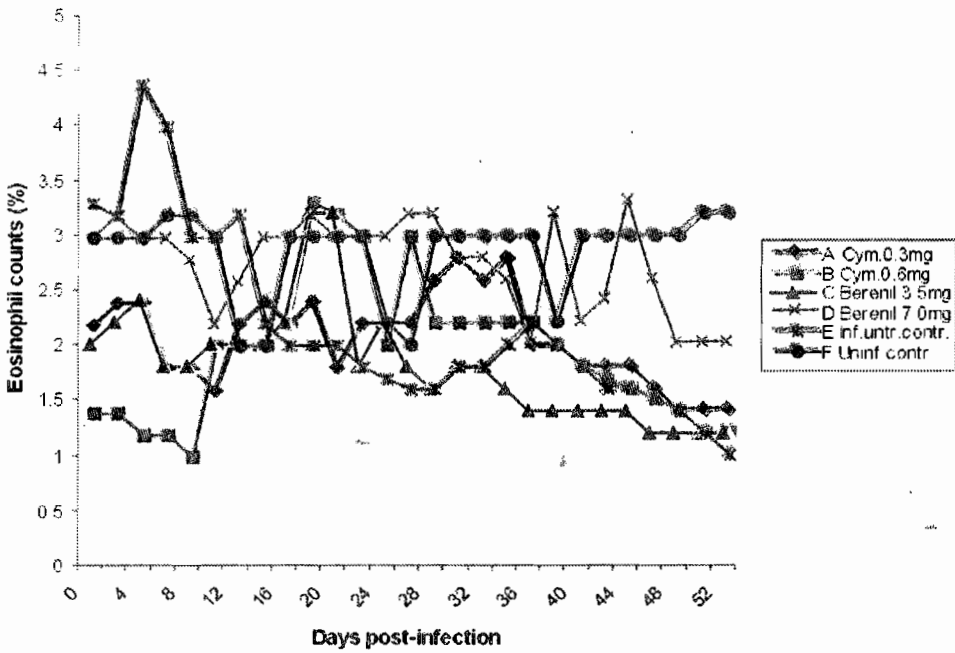
Key: Day of treatment (arrowed)

Fig 10: Mean monocyte counts (%) of red fronted gazelles infected with *T. brucei* and treated with either Cymelarsan® or Berenil® and their controls



Key: Day of treatment (arrowed).

Fig. 11: Mean of neutrophil counts (%) of red fronted gazelles infected with *T. brucei* and treated with either Cymelarsan® or Berenil® and their controls



Key: Day of treatment (arrowed)

Fig 12: Mean eosinophil counts (%) of red fronted gazelles infected with *T. brucei* and treated with either Cymelarsan® or Berenil® and their controls

DISCUSSION

In the present study, where a similar and standard dose of *T. brucei* was administered to all infected groups, might have been responsible for the similar pre-patent period (7 - 8) days observed in all the infected groups. This suggests that the initial parasite replication rates were similar irrespective of host trypanotolerance. Similar observation using the same strain of *T. brucei* was reported in an experimental infection in dogs (Nwosu and Ikeme, 1992). The long prepatent period (7 - 8 days) observed in the wild ungulates in contrast to the usually short prepatent period (2 - 4) days for domestic or laboratory animals might not be unconnected with an attempt by the animals to suppress the infection due to their trypanotolerant trait. A similar prolonged prepatent period with intermittent parasitaemia has been reported, far beyond the expected sequestration period in an experimental *T. congolense* infection of wild waterbucks (*Kobus deffassa*) (Olubayo *et al.*, 1991; Olubayo and Brun, 1992).

The anaemia encountered in all infected groups was associated with a decline in PCV, RBC and Hb concentrations, which corresponded with waves of parasitaemia. Pre-infection values were however attained for Cymelarsan® at 0.3mg and 0.6mg/kg or Berenil® at 7.0 mg/kg body weights. The decline in the value of the RBC indices during periods of parasitaemia, which later maintained a gradual increase during periods of aparasitaemia, shows an inverse relationship between the RBC indices and parasitaemia. This decline in the values of the red blood cell indices was however, effectively modulated to pre-infection values for the gazelles treated with either Cymelarsan® at 0.3mg/kg, 0.6mg/kg or Berenil® at 7.0 mg/kg body weight.

The decline of red cell indices in African animal trypanosomosis is often associated with leucopenia as observed in this study. These observations have been attributed to *in vivo* production of neuraminidase by the parasite during high parasitaemia, which in turn cleave sialic acid of cell membranes therefore contributing to the pathogenesis of red and white cells depletion (Esiebo, 1973). Similarly, the

expanded and active mononuclear phagocytic system (MPS) might have necessitated an increased demand on the system to remove dead blood, tissue cells, trypanosomes and antigen antibody complexes and to participate in immune responses which might have contributed immensely to the destruction of RBC and WBC (Anosa, 1988).

On the other hand, the activation of the MPS might have also been responsible for the consistent monocytosis recorded in the infected gazelles which rose unabatedly in the gazelles treated with Berenil® at 3.5 mg/kg or the infected and untreated control. Stress might have further contributed to the degree of monocytosis encountered in the gazelles. The gazelles were seen to be under intense stress due to the fact that they were subjected to confinement for the first time with a resultant increased blood cortisol level (Mbayo, 2007). Confinement stress in experimental marmosets (*Callithrix penicillata*) has been reported to trigger some degree of monocytosis (Boere *et al.*, 2005).

The lymphopenia observed in this study might have been associated with an increased demand on the system for both immune and inflammatory responses. Similarly, the stress of captivity might have exacerbated the degree of lymphopenia in the gazelles since they were subjected to captivity for the first time. Confinement stress in experimental marmosets (*Callithrix penicillata*) has been reported to cause marked lymphopenia (Boere *et al.*, 2005). The exact cause of a decline in neutrophil numbers was not clear, but splenic sequestration of leucocytes as reported by Anosa (1983) might have prevented neutrophil numbers from rising. Mean while, eosinophil numbers reduced during the early periods of the infection in the infected and untreated controls while other groups experienced slight fluctuations but remained within normal range. Similar observations were experienced in a *T. brucei* infection of dogs, which was attributed to marrow granulocyte hypoplasia (Nwosu and Ikeme, 1992).

The result of the present study indicate that Cymelarsan® at 0.3mg/kg and 0.6mg/kg body weights or Berenil® at 7.0 mg/kg body weight were capable of relieving the typical clinical signs of

trypanosomosis such as elevated body temperature, increased respiration rate, weight loss, starry hair coat and palour of buccal and ocular mucous membranes of red fronted gazelles. The reversal of these clinical signs, which began to amend with the commencement of Cymelarsan® treatment at the onset of parasitaemia, is consistent with earlier reports due to *T. evansi* infections in camels (*Camelus dromedarius*) (Zelleck *et al.*, 1992; Zweygarth *et al.*, 1992) and in captive Asian tigers (*Panthera tigris*) (Parija and Bhattacharya, 2001). There was, however, a graded dose effect with more response being attained with Cymelarsan® at 0.6mg/kg and Berenil® at 7.0 mg/kg. This might further explain why Berenil at 3.5 mg/kg was not very effective probably due to a relapsed infection, which produced a similar trend as the infected and untreated control.

From the foregoing, the lack of relapse or 0% mortality with Cymelarsan® at 0.3mg/kg and 0.6mg/kg body weights in contrast to a relapse with 100% mortality with Berenil® at 3.5 mg/kg body weight and 0% with Berenil® at 7.0 mg/kg body weights, shows that Cymelarsan® might be a better drug of choice in the chemotherapy of trypanosomosis in the red fronted gazelle. Raynaud *et al.* (1991) also proffered the use of Cymelarsan® a less toxic trivalent arsenical than Melarsonyl®, Melarsoprol® or Berenil® in the treatment of reservoir trypanosome infections. Relapsed infection due to Berenil® therapy in domestic animals and laboratory rodents have been reported (Egbe-Nwiyi *et al.*, 2006 and Mbaya *et al.*, 2007). Relapsed infection due to Berenil® therapy has been associated primarily to drug resistance due to under dosage and indiscriminate use of the drug (Onyeyili and Egwu, 1995) or due to the fact that the drug molecules are too large to cross the blood-brain barrier in sufficient quantities to be curative (Jennings, 1991).

The trend of parasitaemia in the infected and untreated control was that of successive waves. This is probably due to the fact that trypanosomes often show antigenic variation, where each peak of parasitaemia consists of mixture of variable antigenic types in different proportions. Most of these variable antigenic types are eliminated by

the host's immune system during remission, leaving a few others to multiply and form the next peak (Barry and Hadjuk, 1979; Nwosu and Ikeme, 1992; Shapiro and Pearson, 1986).

CONCLUSION

Trypanotolerance is not absolute in wild red fronted gazelles subjected to the stress of captivity and severe trypanosomosis similar to that observed in domestic animals may occur in them. The clinico-pathological changes due to trypanosomosis in the gazelles may be arrested by early treatment with either Cymelarsan® (0.3mg/kg, 0.6mg/kg) or Berenil®. It is recommended that additional stress is avoided at all cost when wild animals such as the red fronted gazelles are subjected to any form of captivity in zoos, captive breeding centers or in households.

ACKNOWLEDGEMENTS

The authors wish to thank the Management and Staff of Borno state Wildlife group and Sanda Kyarimi Park, Maiduguri, Nigeria for assistance rendered during the course of the study.

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