INFLUENCE OF NUTRITION ON TRYPANOSOME ISOMETAMIDIUM CHLORIDE CHEMOPROPHYLAXIS

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ABSTRACT

Thirty six weaner wistar rats were used to study the effect of protein nutrition on trypanosome isometamidium chloride prophylaxis. Two groups of rats A and B (n = 18 per group) were maintained on 21% and 14.5% crude protein diet respectively for the twenty eight days. Thereafter, group A was sub-divided into groups A1, A2, and A3 (n = 9) and group B into B1, B2 and B3 (n = 9) respectively. Concurrently, groups A2 and B2 were given isometamidium chloride at the dose of 1mg| kg body weight intramuscularly. Fourteen days later, groups A1, A2 and B1, B2 were inoculated with 10^5 of a low virulent strain of Trypanosoma congolense organisms. Seven days later (before patency), the same groups were re-infected with 10^6 Trypanosoma brucei brucei organisms. Groups A3 and B3 were left as uninfected controls. Prepatent period and parasitaemia were determined using standard techniques. Also rectal temperature and Packed Cell Volume (PCV) were determined using standard methods. Data collected were arranged according to days of activity and group means and standard deviation determined. Comparisons between groups over the course of the investigation were done using one way analysis of variance (ANOVA). There were inconsistent fluctuations in body temperature among all the groups. However, there was a positive correlation between treatment and nutrition on body temperature. The infections caused significant and progressive reduction in PCV ($p<0.05$). Isometamidium treatment appeared to protect against reduction in PCV as well as the parasitaemia profile. Generally, the HPD group first appeared more vulnerable but as the infection became chronic, more serious pathology was observed among the untreated LPD group. Within the limits of our design, there were interactions between the drug and nutrition in the pathogenesis and pathology of T. brucei brucei infection.

Keywords: Dietary protein, Trypanosome infection, Isometamidium chloride, Chemoprophylaxis

INTRODUCTION

Animal trypanosomiasis encompasses a variety of disease syndrome caused by trypanosomatid parasites in various animal species. The disease has been considered one of the major obstacles to livestock production in Africa (Wilson, *et al.,* 1983; Holmes *et al*., 2000). The animal trypanosomes include *T. vivax*, *T. congolense*, *T. simiae*, *T. b. brucei*, *T. evansi* and *T. equiperdum* each of which affects one or more animal species. Susceptible hosts range from cattle, sheep, goats, pigs, horses, camels dogs, and to cats. Many wild species are susceptible and these tend to act as reservoirs of infection. The classical clinical manifestation of infection is anaemia characterized by decreased packed cell volume (PCV), haemoglobin (Hb) level, and red

blood cell (RBC) counts. White blood cell (WBC) count may decrease or increase (Anika *et al*., 1987; Anene, 1987; Onyeyili and Anika, 1989). Other symptoms include pyrexia, anorexia, and loss of condition and death (Anosa, 1981).

Chemotherapeutic agents are used for the treatment and control of the animal trypanosomosis. However, since the introduction of has Isometamidium bromide (Samorin^{R}) in 1961 (Berg *et al.,* 1961), little further progress have been made in the development of animal newer trypanocide. This slow rate of development of newer trypanocides which has been compounded by development of resistance to the available drugs by the parasites as well as cases of relapsing parasitaemia has led to an impasse in the chemotherapy of trypanosomosis. The presence of an uncontrolled environment containing tsetse flies and wild reservoir hosts is another limiting factor in the control of the disease (Uzoukwu, 1981)

Chemotherapeutic treatment of livestock is not usually advocated in trypanosome endemic areas unless a strict treatment and dosage regimen can be assured (WHO, 1979), since no prophylactic or therapeutic effect can be achieved once the parasites become resistant to the drug (Sutherland *et al.,* 1991). However, chemoprophylaxis may be indicated in endemic areas to protect animals on farms and ranches, livestock that seasonally move into tsetse infested areas provided that this is carried out as soon as the animals are back from trypanosome free areas in case of the latter, and trade and slaughter animals that must travel through tsetse infested areas ((Finelle, 1973; Aliu, 1991). Furthermore, it has been shown that with good husbandry and regular prophylaxis, animals can thrive in areas of moderate to high tsetse challenge (Trail *et al.,* 1986).

Centuries of exposure to trypanosome organisms have produced breeds of livestock in tsetse endemic areas that exhibit some degree of tolerance to the infection (Dwinger *et al.,* 1992).This phenomenon called trypanotolerance which differs among breeds have also been shown to be influenced by a number of factors among which the nutritional status was considered most important (Murray, 1987; Reynolds and Ekwuruke, 1988). Allonby (1974) had earlier observed in ovine haemonchosis, that the infection was severer during dry season, a period of harsh nutritional insult. In cattle trypanosomosis, dietary supplementation reduced the severity of the infection as shown by apparently stable PCV (Agyemang *et al.,* 1990; Little *et al.,* 1991). The role of nutrition is believed to be due to its provision of substrates necessary for adequate immuno responsiveness and neuroendocrine control of homeostasis in the host (Norton *et al.,* 1986). Generally, malnutrition and Protein deprivation have been shown to down regulate immune response and resistance to infections (Scrimshaw *et al.,* 1968; Chandra, 1984).

Isometamedium bromide is one of the most commonly used trypanocide and one that is ascribed the potency of prophylaxis (Ezeokonkwo *et al.,* 2004). The drug is used during periods of feed abundance and scarcity corresponding to rainy and dry seasons for ruminants and at all times for pets (cats and dogs) and horses in endemic areas. The clearly known effect of starvation on the course of the disease such as HIV AIDS and Cryptosporidiosis (Grimble, 2001; Keusch, 2003) creates a possibility (at imagination level) of different responses to the drug given at different periods of nutritional resource availability. Hitherto, no study has reported the effect of nutrient abundance or starvation on the trypanosome chemoprophylaxis as a means of ascertaining the efficacy of such programmes. It is therefore the aim of this study to investigate the possible influence of nutrition on trypanosome chemoprophylaxis.

MATERIALS AND METHODS

Laboratory Animal Management: A total of thirty six weaner wistar rats of 150 ± 11.1 g mean live-weight, procured from the Laboratory Animal Unit of the Benue State University were used to investigate the effect of dietary protein on isometamedium trypanosome chemoprophylaxis. These were kept in grated metal rat cages and housed in our Departmental

mini- laboratory animal house for two weeks for acclimatization both to the environment and feeding regimen. During this period they were fed iso-protein and iso-energetic diet with water provided *ad libitum* with sufficient effort aimed at avoiding undue discomfort and conforming to the standard ethics on the use of laboratory animals.

Post adaptation, they were randomly divided into two equal groups A and B (n = 18). Each group was comfortably housed in two large cages each containing 9 rats. Two diets, A and B were formulated and members of group A were fed diet A, while those of group B were fed diet B. These diets differed mainly in their dietary protein content (Table 1). In both cases feeding was *ad libitum* and lasted till the end of the study.

Table 1: Proximate analysis of the two dietary types

Dietary components	High protein diet (%)of ingredients	Low protein diet (%) of ingredients
Crude protein	21	14.5
Fat	8.5	7.0
Crude Fibre	6.0	7.0
Calcium	1.2	1.2
phosphorus	0.45	0.45
Metabolisable energy	2800Kcal\kg	2750Kcal\kg

Experimental Design: The post acclimatized two groups, were maintained on their respective diets for 28 days. Thereafter, groups A and B were sub-divided into three equal groups, A_1, A_2, and A_3 and B_1, B_2, and B_3 respectively with each triplicate housed separately. Groups A_2 and B_2 rats were inoculated intra-muscularly with a freshly prepared solution of isometamidium bromide (Samorin^r) (1mg\kg body weight). This treatment was not given to A_1, B_1 or A_3, B_3 rats. Two weeks later, rats in groups A_1, B_1, and A_2, B_2, were infected with 10^5 *Trypanosoma congolense* organisms (untyped strain) known to be of low virulence. Seven days later, when the infection has not shown patency, the rats in groups A_1, B_1 and A_2, B_2 were re-infected with 10^6 virulent *T. brucei* (Federer strain). Groups A_3 and B_3 were left as uninfected controls.

Prepatent Period and Parasitaemia: Two days post inoculation with *T. congolense*, blood was collected from each member of the infected group through a snip at the tip of the tail directly on to a clean microscopic slide. This was covered with a cover slip and a thorough examination for trypanosome organisms made under a microscope as described by Herbert and Lumbsden (1976). This was continued daily for five days when the second infection with *T. brucei brucei* was given. The prepatent period determined as the time lapse between infection and appearance of trypanosome organisms in blood. Parasitaemia was also concurrently determined for the various groups and this was continued till day 14 after the secondary infection using the same procedure.

Body Temperature and Packed Cell Volume: A day prior to parasite inoculation, body temperatures of members of each group was determined through rectal insertion of clinical thermometer followed by the determination of group means. This was repeated on the day of infection and daily thereafter, till the end of the study. Concurrent with body temperature determination, members of each dietary group were bled through a snip at the tail tip, blood was collected directly into heparinised bottles and this was used to determine the PCV using the method of Dacie and Lewis (1995), followed by the calculation of the group means and standard deviation.

Treatment of the Infected Animals with Isometamidium: Two weeks after the secondary infection, members of all the infected groups were treated with isometamidium chloride at the dose rate of 1mg\kg body weight. Wet mount of fresh blood was used to monitor the profile of the restoration of PCV and disappearance of parasitaemia.

Statistics: The daily and weekly data for the various parameters were subjected to one-way Analysis of Variance (ANOVA) in order to find out interactions between them and over time. Significant means were separated by least significant difference (LSD) (Duncan, 1986).

RESULTS

Body Temperature: The various groups did not manifest any consistent body temperature pattern throughout the period of investigation. There were temperature fluctuations among all the groups. The control groups exhibited significantly higher mean body temperatures relative to their infected counterparts ($p < 0.05$). However, within dietary groups there was interaction between diet and body temperature with contrasting values between groups. Thus group B_1 on poor diet and without prophylactic medication was more prone to the infection, induced hyperthermia, relative to the protected B_2 ($p < 0.05$), while among the high dietary protein group, group A_2 that received prophylactic medication had significantly higher mean body temperature relative to A_1 ($p < 0.05$). Our results also showed that high protein diet appeared to predispose animals to higher body temperature whether infected or not (Figure 1).

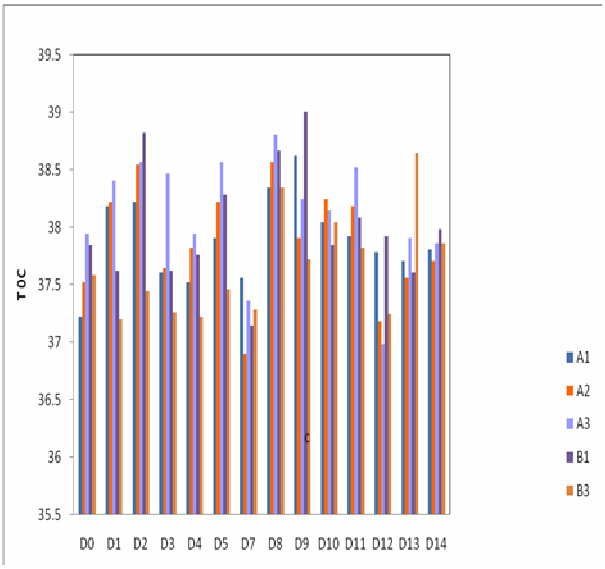

Figure 1: Variations in body temperature among the various groups

Packed Cell Volume: There was significant ($p < 0.05$) reduction in PCV irrespective of diet or prior chemoprophylactic medication. This infection induced anaemia was also progressive over time. There was an initial heightened response by the high protein diet group to anaemia relative to those on poor protein diet. This was however not sustained as the PCV value for the unprotected B1 fell significantly ($p < 0.05$) below those of A_1, A_2 as well as B_2 as the infection progressed. Prophylactic medication in addition appeared to protect against infection induced reduction in PCV significantly ($P < 0.05$) as was clearly shown at week 2 of infection. Generally, protein nutrition appeared to have negatively correlated with the PCV especially among the poor dietary protein fed infected B_1 (Figure 2).

Parasitaemia: Group A_1 exhibited the shortest prepatent period of 7 days. Between days 10 and 11 pi, all the groups had shown patency with group A_1 having significantly ($P < 0.05$) higher parasitaemia relative to the other groups, a position that was upheld till day 15 pi (Figure 3). There was also a generalized and progressive parasitaemia in all the infected groups. Prophylactic medication caused reduction in parasitaemia in both dietary groups compared to groups that received no protection during the first wave of rising parasitaemia. This was clearly manifested between days 18 and 21 among the low protein diet group (Figure 3). Also, the treated high protein diet group showed significantly lower parasitaemia ($p < 0.05$), relative to its unprotected counterpart. Isometamidium chloride appeared to have protected the animals in the low dietary protein group as the untreated group exhibited significantly ($p < 0.05$) higher parasitaemia relative to the treated group (Figure 3).

Recovery Post Treatment: The disappearance of parasitaemia was similar in both dietary and treatment groups and this was within twenty four hours. Similarly, the profile of the restoration of PCV post treatment was comparatively the same in all the groups.

DISCUSSION

We have in this study attempted a possible integration approach (chemotherapy and management) in the control of one of the most important livestock diseases plaguing sub-Saharan Africa.

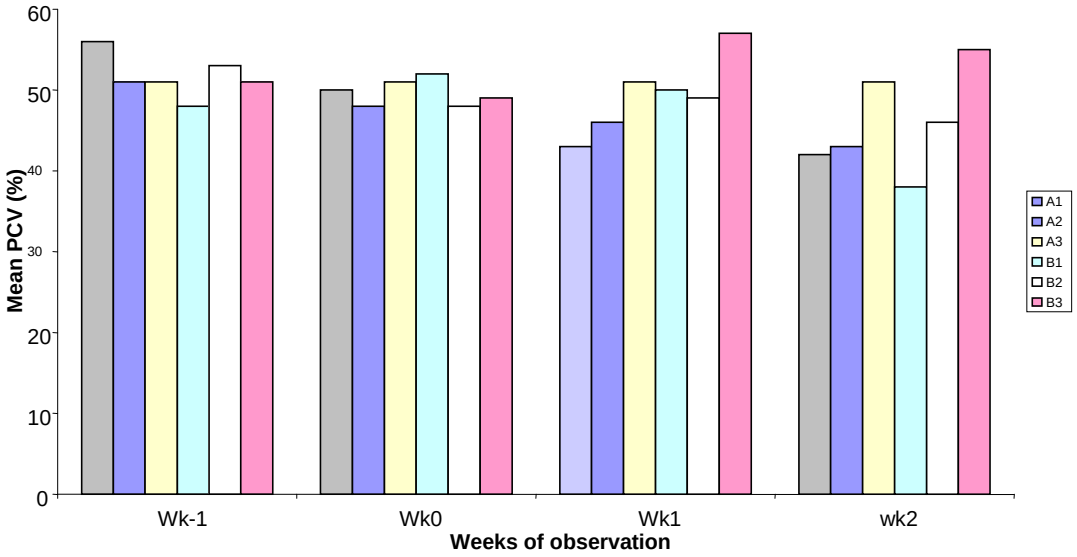

Figure 2: Variations in PCV among the various groups over time

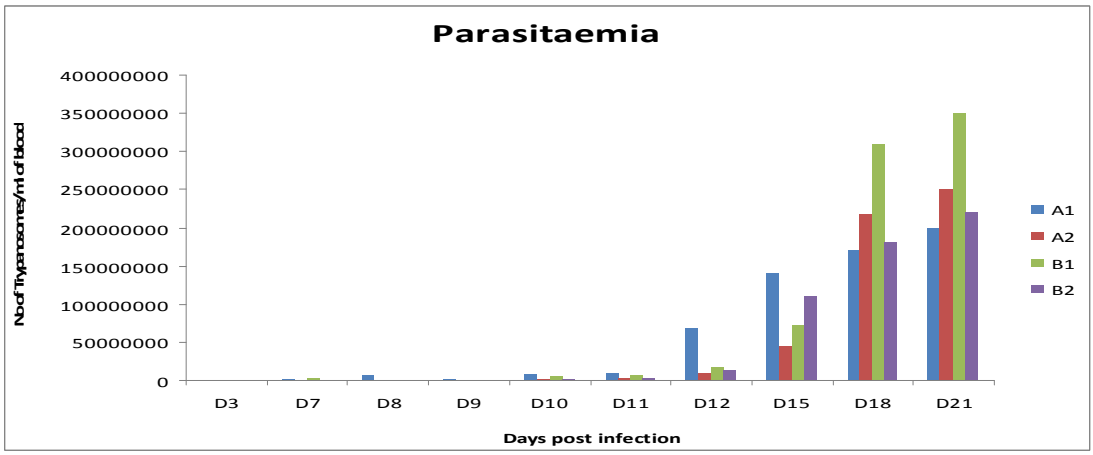

Figure 3: Parasitaemia profile among the infected groups

The fluctuating body temperature as recorded in this study is characteristics of animal trypanosomosis (Stephen, 1986). The development of pyrexia during trypanosome infection has been attributed to the parasite induced increased metabolic rate in the host (Holmes *et al.,* 2000). Trypanotolerance (a situation among some indigenous breeds of cattle sheep and goats in the forest zones of West Africa that can habour trypanosome infection without apparent clinical signs) is also associated with the absence of fever during the course of the infection (Holmes *et al.,* 2000). The present study contrasts earlier report regarding pyrexia in infected hosts (Holmes *et al*., 2000; Nnadi *et al.,* 2010). Nnadi *et al.* (2010) observed that pigs infected with trypanosomes irrespective of diet experienced significant hyperthermia, although the controls had higher body temperatures in some instances. In the present study, pyrexia was associated with rising waves of parasitaemia and shows lack of parasite inhibition irrespective of diet. Also, there was no interaction between prophylaxis and body temperature except at the prepatency stage when the treated animals had lower body temperature relative to the untreated. Post patency, this property appeared to have been lost possibly due to the decreasing blood drug level with time. One noteworthy observation in this study is the apparent higher body temperature by the groups fed high protein diet. It is suggested that this may be due to higher digestible energy content relative

to that of the poor protein diet. It could again be that the tryptophol that is associated with pyrexia above is related to the dietary quality of tryptophan in the diet.

Parasite establishment and the development of parasitaemia were affected by the quality of the diet. This contrasts with the demonstrated effect of high dietary protein on protozoan and helminth parasites establishment (Reynolds and Ekwuruke, 1988; Little *et al.,* 1990; Nnadi *et al.,* 2007) but agreed with the report of Otesile *et al.* (1991). In this study, high protein diet appeared to enhance both establishment and early multiplication of the parasites. Generally, there are conflicting reports on the role of nutrients on the pathogenesis of protozoan haemoparasites. Earlier studies in rodent malaria (Edirisinghe *et al*., 1981; Fern *et al.,* 1984; Logan *et al.,* 1984; Edirisinghe, 1986) showed that acute and chronic protein deprivation depressed peak parasitaemia in *Plasmodium* infected rats. It has been demonstrated that there was increased plasma levels of phospholipids and cholesterol in trypanosome infected sheep fed high protein diet (Katunguka-Rwashikaya, 1997). This may be responsible for the higher parasitaemia among the groups maintained on high protein diet as cholesterol has been shown to supports the growth and differentiation of trypanosomes (Katunguka-Rwashikaya *et al.,* 1993). There was also an initial protective influence of the isometamidium on parasitaemia. This appeared to wane over time such that, as the infection was prolonged the protection ceased to be evident especially among those on high protein diet. However, protection by the prophylactic agent lasted longer among those maintained on poor protein diet as their unprotected counter parts experienced more progressive and sharper rising parasitaemia. It was expected that nutritionally stressed animals should have shorter periods of protection than those without any insult. It has been suggested that the plasma protein level especially albumin fraction whose level is known to be lowered as a result of the infection may be responsible (*Nnadi et al.,* 2010). This is because of its function in the transport of and bioavailability of drugs. Thus, the altered state of protein malnutrition may have resulted in an altered pharmacokinetics of the isometamidium chloride (Connor, 1998)

The infection induced reduction in PCV, irrespective of nutritional regimen agreed with the results of earlier studies in cattle (Agyemang *et al.,* 1990), and in pigs (Nnadi *et al.,* 2010). However, it contrasted the reports of Little *et al.* (1990), Akinbamijo *et al.* (1997) and Bennison (1997) in cattle, Fagbemi *et al.* (1990) and Makinde *et al.* (1991) in pigs, Zwart *et al.* (1991) in goats and Katunguka-Rwashikaya *et al.* (1993) in sheep. There are conflicting opinions regarding the role of dietary protein in the pathogenesis of trypanosome induced anaemia. Katunguka-Rwashikaya *et al*. (1993) showed that protein supplementation in an infected sheep produced microcytic anaemia as opposed to normoblastic anaemia in protein deprived hosts. It appeared from their findings that protein acted as a nutrient in haemopoiesis. However, Makinde *et al.* (1991) demonstrated an increased plasma volume in protein deprived infected pigs as opposed to normal plasma volume in protein supplemented ones. The possible mechanism of increased plasma volume in protein malnutrition should be the relationship between the plasma albumin level and normo-hypo and hyper volaemia. This study did not show a correlation between PCV and diet, though the higher parasitaemia by the high dietary protein groups correlated with PCV.

Chemoprophylaxis showed a protective effect on the PCV and this was more pronounced among the group undergoing protein insult. This may be due to the serum level of the drug which may be high due to reduced biodegradation in the liver and slower elimination as explained earlier. The absence of parasitaemia three days post treatment in both dietary groups demonstrated the efficacy of isometamidium chloride that is not subject to the hosts nutritional regimen.

In conclusion, this study showed that protein nutrition influenced the efficacy of isometamidium chloride and the possible duration post administration beyond which the protection from the drug may not be anticipated. Moreover, we suggest that further investigation be carried out to establish the relationship between hepatic damage in

trypanosomosis and hepatic enzymes, dietary protein and serum albumin level on the pharmacokinetics of the drug.

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