

## OBSERVATIONS ON THE TOLERANCE OF YOUNG DOGS (PUPPIES) TO INFECTION WITH *TRYPANOSOMA CONGOLENSE*

Abenga, J. N., David, K., Ezebuio, C. O. G., Lawani, F. A. G.

Nigerian Institute for Trypanosomiasis Research,  
PMB 2077, Kaduna, Nigeria

Correspondence to: Dr. Jerry N. Abenga

Studies were undertaken to assess the susceptibility of young local dogs to infection with *Trypanosoma congolense*. Six puppies (7 weeks old) were used for the study. Although the puppies became parasitaemic 6 to 7 days post infection, they were tolerant to infection as the parasitaemia remained low through out the first seven weeks of the eight week observation period. The packed cell volume (PCV) also only dropped slightly during the last four weeks attaining the value of  $25.6 \pm 3.8$  ( $p > 0.05$ ) by the eighth week while the mean body weight continued to increase. Similarly, the mean daily body temperature did not differ significantly from those of un-infected control. The significance of trypanotolerance in Nigerian local dogs is discussed.

**Keywords:** local puppies, low parasitaemia, packed cell volume, *Trypanosoma congolense*, trypanotolerance, Nigerian

### INTRODUCTION

Trypanotolerance has been described as the relative capacity of an animal to control the development of the trypanosome parasite and to limit their pathological effects, the most prominent of which is anaemia (1-3). Natural resistance to trypanosomes and trypanosomosis is genetically determined (4). The decreasing efficacy of available trypanocidal drugs and the difficulty of sustaining tsetse control have increased the imperative need to enhance trypanotolerance through selective breeding either within breeds or through cross breeding (1, 5).

In West Africa, shorter breeds of cattle, N'Dama and Muturu and West African Dwarf (WAD) sheep and goat (6) are known to be trypanotolerant while not much is known about the tolerance status of the different breeds of local dogs. Beside, the known epizootiological roles dogs' play in the spread of African trypanosomosis in animals (7), canine trypanosomosis, is a devastating disease resulting to anaemia, infertility,

abortions and death if not treated (8). Identification of trypanotolerant trait in breeds of Nigerian local dogs may be an effective tool in the control of disease in dogs.

Pathogenic trypanosome species infective to dogs include *T. congolense*, *T. brucei* and *T. evansi* (9). Dogs are also known to be readily infected by human infective *T. gambiense* (7) and *T. rhodesiense* (9). The disease caused by *T. congolense* may be both severe and fatal in dogs (8). In this study, we report clinical manifestation of trypanotolerance in young Nigerian local puppies infected with *T. congolense*.

### MATERIALS AND METHODS

#### *Experimental animals*

A total of six (6) local puppies of 7 weeks old made up of 2 males and 4 females weighing 2.0 to 3.2kg body weight (BW) were used. All of the six dogs were both welped by the same mother at the Nigerian Institute for Trypanosomiasis Research quarters in Kaduna. The bitch was a local dog and

mounted by other local dogs within the area. The puppies were first of all acclimatised in their kennels at the institute laboratories for one week before use. During this period, they were de-wormed with Piperazine citrate, Dicestal<sup>(R)</sup> and Dinitrophenol against roundworms, tapeworms and hookworms respectively. They were regularly deticked with Diazinon, Diazintol, <sup>(R)</sup> at 2weeks intervals. The diet was made of milk, beans, rice, yams, vegetables, soybeans and meat occasionally. Water was provided *ad libitum*.

#### **Trypanosome parasites**

Parasites used were *T. congolense* (NITR/Federe) isolated from cattle and cryopreserved in liquid nitrogen from where they were later sub-passaged once, into albino rats before use.

#### **Experimental design and sample analysis**

Four (4) of the puppies, Nos. 01, 02, 04 and 05, were randomly selected and infected with  $1.0 \times 10^6$  of the parasites subcutaneously. The remaining 2 puppies; Nos. 03 and 06 served as uninfected control. Wet blood film taken daily from the ear vein of the infected puppies was used for estimation of parasitaemia under the x40 objective of the light microscope. 100 microscopic fields were examined before the result was declared negative.

Blood for estimation of Packed Cell Volume (PCV) and other haematological values was obtained through venipunctures of the femoral vein using 21 gauge hypodermic needle and 5mls syringes. Capillary tubes were 3/4 filled with the whole blood, sealed one end with plasticine and centrifuged for 5 minutes

in a microhaematocrit centrifuge at 12,000G. The PCV was read off the haematocrit reader (10). Whereas daily rectal temperatures were obtained with the help of the clinical thermometer, body weight of the puppies was obtained weekly using a balance (Henson<sup>(R)</sup> Gallenkamp, England). The data was analysed using student t-test.

#### **RESULTS**

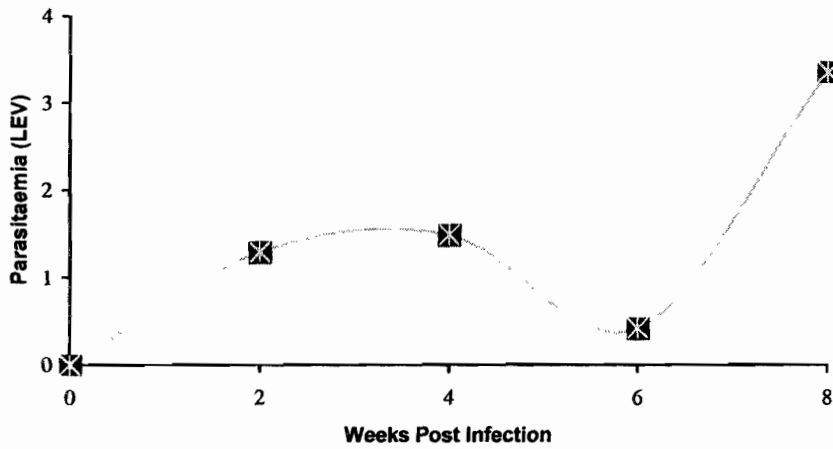
The young puppies became parasitaemic with *T. congolense* 6 to 7 days post infection (PI). However, parasitaemia remained low with mean log equivalent value (LEV) of  $1.07 \pm 0.57$  which lasted for seven weeks but only increased to  $3.36 \pm 0.32$  ( $P < 0.05$ ) by week 8 PI (Fig. 1).

The Packed Cell Volume of infected dogs continued to increase from the mean pre-infection value of  $27.85 \pm 0.21\%$  for the first 4 weeks of infection attaining maximum value of  $31.00 \pm 1.68\%$  by week 3 PI and later declined in the last 4 weeks to the value of  $25.6 \pm 3.81(\%)$  by week 8 PI ( $P > 0.05$ ; Fig 2), while the values of uninfected control continued to rise. The over all changes in the mean PCV values of control and infected dogs did not differ from each other (Table 1).

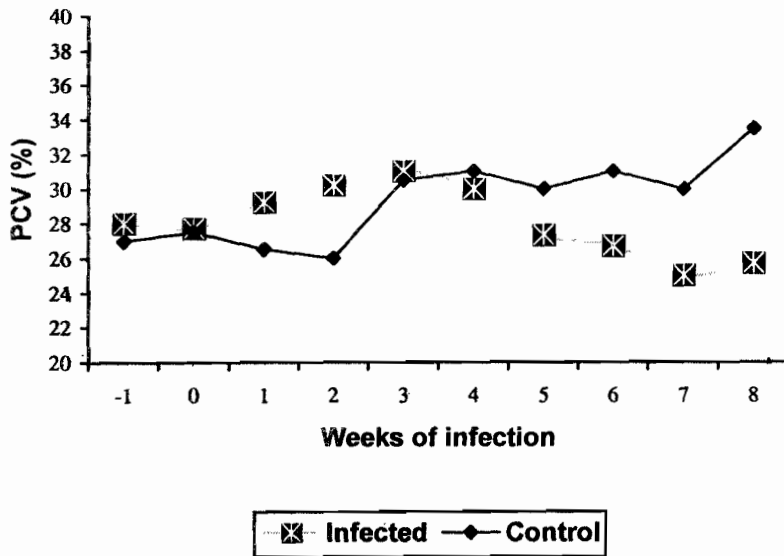
The mean average body weight of the *T. congolense* infected dogs was not adversely affected as it continued to increase form pre-infection value of  $2.6 \pm 0.14$  kg attaining the maximum weight of  $4.8 \pm 0.7$ kg by week 8PI (Fig 3), when the experiment ended. The overall changes in the mean daily body temperature of infected puppies also did not differ from those of control puppies ( $P > 0.05$ , Table1).

**Table 1: Summary of overall changes in the mean value of packed cell volume, body temperature and body weight of control and *T. congolense* infected puppies.**

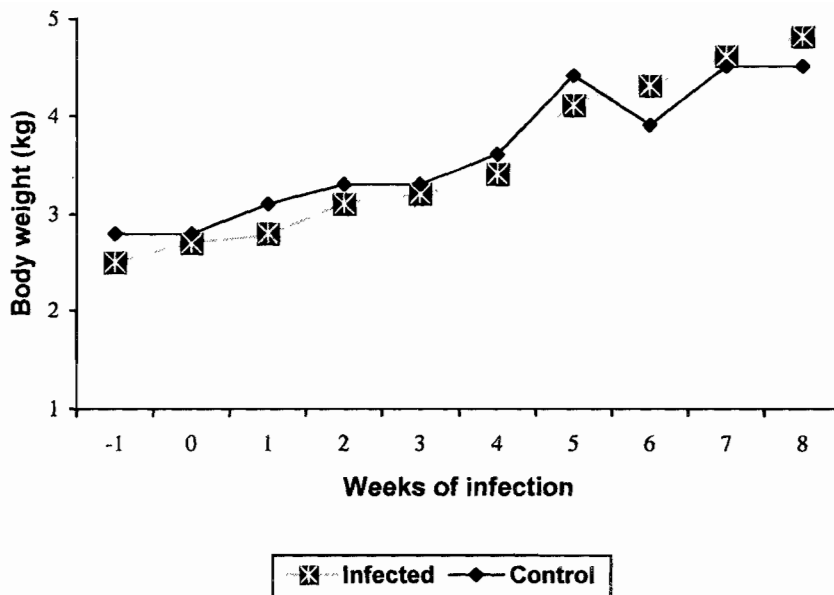
	Control	Infected	
		Pre-infection	Post-infection
Packed Cell volume (%)	29.2±2.42	27.85±0.21	28.13±1.15
Body temperature (°C)	38.01±1.09	38.5±0.28	38.71±0.43
Body weight (Kg)	3.63±0.67	2.60±0.14	3.79±0.75



**Fig 1: Mean Parasitaemia (LEV) of puppies infected with *T. congolense***



**Fig 2: Packed Cell Volume (%) of *T. congolense* - infected and control puppies**



**Fig 3: Changes in the Mean body weight (Kg) of *T. congolense* - infected and control puppies**

## DISCUSSION

Innate ability of trypanosome infected animals to control anaemia and development of parasitaemia have been identified as indicators of trypanotolerance (2). The parasitaemia of *T. congolense* infected puppies observed here was relatively low for seven weeks and increased only by the eighth week. This is at variance with the fulminating parasitaemia observed in *T. congolense* infected trypanosusceptible mice (11) and Nigerian Yankassa sheep (12) in which the animals died within one week and in less than 4 weeks PI respectively. The ability of the young dogs to control parasitaemia within the 8 weeks observation period indicates genetically determined tolerance to *T. congolense*.

Similarly, the Packed Cell Volumes of the infected puppies did not fall

significantly throughout the period indicating innate ability to control development of anaemia (1, 2). These observations compare well with those of *T. congolense* infection in trypanotolerant sheep and goats (13) in which infected animals exhibited chronic anaemia and were able to control parasitaemia over a long period. Omotainse (14) however reported susceptibility of adult dogs to *T. brucei* infection resulting to death of some of the dogs 5 to 42 days post infection after developing low PCV.

Trypanotolerance is a genetically determined complex mechanism involving factors which are not yet well known. Naessens *et al* (3) reported two mechanisms involved in natural resistance to African trypanosomosis in cattle in West Africa,

namely; an innate mechanism that controls parasite growth, and another involving haemopoetic system that is able to limit anaemia. The low parasitaemia observed in *T. congolense* infected puppies may thus not be unconnected with ability of the dogs to control parasite development due to trypanolytic factors in the serum of infected dogs as has been demonstrated by Wang *et al* (15) in *T. brucei* and *T. congolense* infected cape buffalos. This has been shown to involve two factors; namely, complement-dependent and clone specific lytic activity and, complement-independent trypanocidal activity that are not restricted to trypanosome clones and species (15).

Similar anti-trypanosomal activity was demonstrated in the sera obtained from Cape buffalo, giraffe and greater Kudu resulting to inhibition of replication of *T. brucei* (16). Also, serum xanthine oxidase, serum catalase and trypanosome specific immune responses have been reported to play roles in regulation of the level of trypanosome parasitaemia in trypanotolerant Cape buffalo (17). Logan-Henfrey *et al* (18) reported that the bone marrow response is a key determinant factor of trypanotolerance in cattle as it determines the animal's capacity for haematopoietic cell regeneration and control of anaemia. This was supported by light and electron microscopic studies of sequential biopsies of bone marrow which showed key differences between trypanotolerant N'dama and trypanosusceptible Boran cattle. Such

mechanism may also have been responsible for the low anaemia in the *T. congolense* infected puppies.

Almost normal Packed Cell Volume, progressive increase in body weight and normal body temperature observed in the infected young dogs confirmed the limited pathological effect of *T. congolense* on the puppies. Whereas further studies may be needed to confirm the trypanotolerant traits in Nigerian local dogs, the ability of the *T. congolense* infected puppies to resist parasitaemia and development of anaemia are indicative of trypanotolerance in the local puppies.

Trypanotolerance in local dogs may serve as an important measure against the current upsurge in cases of canine trypanosomosis in the country and limit mortality of exotic breeds of dogs through crossbreeding with the tolerant breeds.

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