VETERINARSKI ARHIV 75 (2), 165-174, 2005

Studies on anaemia in Nigerian local puppies infected with *Trypanosoma congolense*

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ABENGA, J. N., C. O. EZEBUIRO, K. DAVID, A. O. FAJINMI, S. SAMDI: Studies on anaemia in Nigerian local puppies infected with *Trypanosoma congolense*. Vet. arhiv 75, 165-174, 2005.

ABSTRACT

Investigation into the effect of infection with *Trypanosoma congolense* on the haematology of growing Nigerian local dogs was undertaken using 6 puppies infected with 1 x 10^6 of the parasites. Infection resulted in mild anaemia characterized by a slight drop in the Packed Cell Volume (PCV), Haemoglobin (Hb) and Red Blood Cells (RBC) counts which did not occur until the last half of the 8-week observation period. The anaemia was macrocytic normochromic. The mild decrease in the overall erythrocyte values of *T. congolense*-infected young dogs was attributable to trypanotolerance in the local breed of dog. However, the infected group did not attain full erythrocyte values as in the control group, suggesting that similar changes occurring in infected young animals contribute to retarded growth associated with trypanosome infections.

Key words: anaemia, dogs, *Trypanosoma congolense*, trypanotolerance

Introduction

African animal trypanosomosis constitutes a major impediment to livestock production and economic development in several parts of sub-Saharan Africa, including Nigeria (SWALLOW, 2000; ABENGA et al., 2002; MASIGA et al., 2002), despite decades of attempts to control the disease and its vectors (MOLYNEUX, 2001). Canine trypanosomosis is a devastating disease leading to anaemia, infertility, abortions and death if not treated (LOSOS and IKEDE, 1972).

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ISSN 0372-5480
Printed in Croatia

Whereas *T. vivax, T. congolense* and *T. brucei* are the major causes of disease in ruminant, *T. congolense, T. brucei* and *T. evansi* (STEPHEN, 1970) cause disease in dogs. Dogs are also known to be infected by human infective *T. gambiense* (GIBSON et al., 1978) and *T. rhodesiense* (STEPHEN, 1970). In Nigeria, trypanosomosis in dogs due to *T. congolense* occurs commonly (ANENE et al., 1999).

Although several advances have been made on research into the various aspects of the pathogenesis and pathology of the disease, the exact factors involved in the disease process are not yet fully known. In many endemic areas young animals may be exposed to the same risk of infection with trypanosomosis as adult animals. However, less attention has been paid to the impact of the disease on growing animals. This may have been responsible for the lack of sufficient knowledge necessary to control the present huge economic losses arising from the mortality of young animals infected with trypanosomosis.

In this study we report our investigation into the effects of infection with *T. congolense* on the erythrocyte values of growing local Nigerian dogs.

Materials and methods

Experimental animals. A total of six 7-week-old local puppies of mixed sexes, weighing 2.0 to 3.2 kg body mass, were used.

All 6 dogs were whelped by a single mother at the Nigerian Institute for Trypanosomiasis Research centre in Kaduna. The bitch was a local dog and mounted by other local male dogs from within the area. The puppies were acclimatized for one week at the Institute Laboratories before use. During this period they were dewormed with piperazine citrate Dicestal® and Dinitrophenol against round worms, tape worms and hook worms, respectively. They were regularly deticked at 2 week-intervals using Diazintol®. The dogs were fed with milk, beans, rice, yams, soya bean meal, and occasionally meat, while water was provided ad libitum.

Trypanosome parasites. Trypanosoma congolense (NITR/Federe) isolated from cattle in Federe in Kaduna State, Nigeria, was used for the study. The parasites were cryopreserved in liquid nitrogen, from where they were sub-passaged into donor albino rats prior to use.

Experimental design. Four of the dogs, numbered 01, 02, 04 and 05, randomly selected to form the infected group, were each inoculated with 1×10^6 of the parasites via the subcutaneous route. The remaining 2 dogs, N^o 03 and N^o 06, served as an uninfected control group.

Sample collection and analysis. Wet blood film made daily from the ear vein of the infected puppies was used for estimation of parasitaemia. However, the blood for haematology was obtained through venipuncture of the cephalic vein using 21-gauge

hypodermic needles and 5 ml syringes. The blood was collected into ethylene diamine tetra acetic acid (EDTA) bottles prior to use.

To obtain the PCV of the dogs, capillary tubes were l' filled with whole blood, sealed at one end with plasticine and centrifuged for 5 minutes in a microhaematocrit centrifuge at 12,000 g and the PCV read off the haematocrit reader as described by KELLY (1979). Haemoglobin concentration was estimated using the cyanomethaemoglobin method. The RBC counts were also enumerated as described by KELLY (1979).

Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) for each dog was also calculated. Data obtained was analyzed statistically using Student's *t*-test.

Results

Trypanosoma congolense - infected puppies became parasitaemic 6 to 7 days post-infection (PI). The sequential changes in the mean erythrocyte values of the dogs are shown in Table 1 and Fig. 1. The PCV of the dogs before infection was 27.7 ± 0.5%. After infection the values increased for the first half of the 8th week of the observation period, attaining the maximum of 31.0 ± 1.7% by the 3rd week PI. It then declined progressively in the remaining 4 weeks to values slightly below that of pre-infection, attaining a value of 25.6 ± 3.8% ($P>0.05$) by week eight. A similar pattern was observed in the changes in Hb values. The mean Hb level of infected dogs increased from a pre-infection value of 8.9 ± 0.1 gm/dl for the first 4 weeks of infection to the maximum value of 10.0 ± 0.6 gm/dl by week three, and then declined from the fifth week to values slightly below pre-infection values for the remaining weeks of the observation period. Mean pre-infection RBC count of the dogs was 4.45 ± 0.6$10^6$/μl but increased slightly by week 1 PI, thereafter dropping to slightly below pre-infection values for three weeks. Further decreases in RBC values occurred in the last 4 weeks of the infection, reaching values of 2.6 ± 0.6×10^6/μl and 2.4 ± 1.0×10^6/μl ($P<0.05$) by weeks 7 and 8 PI, respectively. However, the values of the control dogs fluctuated between normal ranges (Fig. 1).

The mean corpuscular volume of infected puppies initially decreased slightly in the first week of infection from the pre-infection value of 66.1 ± 0.9 fl but thereafter showed a fluctuating increase in the remaining weeks of infection, attaining a maximum value of 85.9 ± 1.2 fl ($P<0.05$) by week 7 PI. The pre-infection mean corpuscular haemoglobin value of the dogs was 23.6 ± 5.9 pg. After infection the values decreased slightly in the first two weeks of infection but thereafter fluctuated slightly above pre-infection values in the remaining weeks, with a sporadic increase to 33.2 ± 7.8 pg ($P\leq0.05$) by week 6 PI.

The pre-infection mean corpuscular haemoglobin concentration of the puppies was 32.1 ± 0.1 gm/dl, but only fluctuated within the same pre-infection ranges, showing only

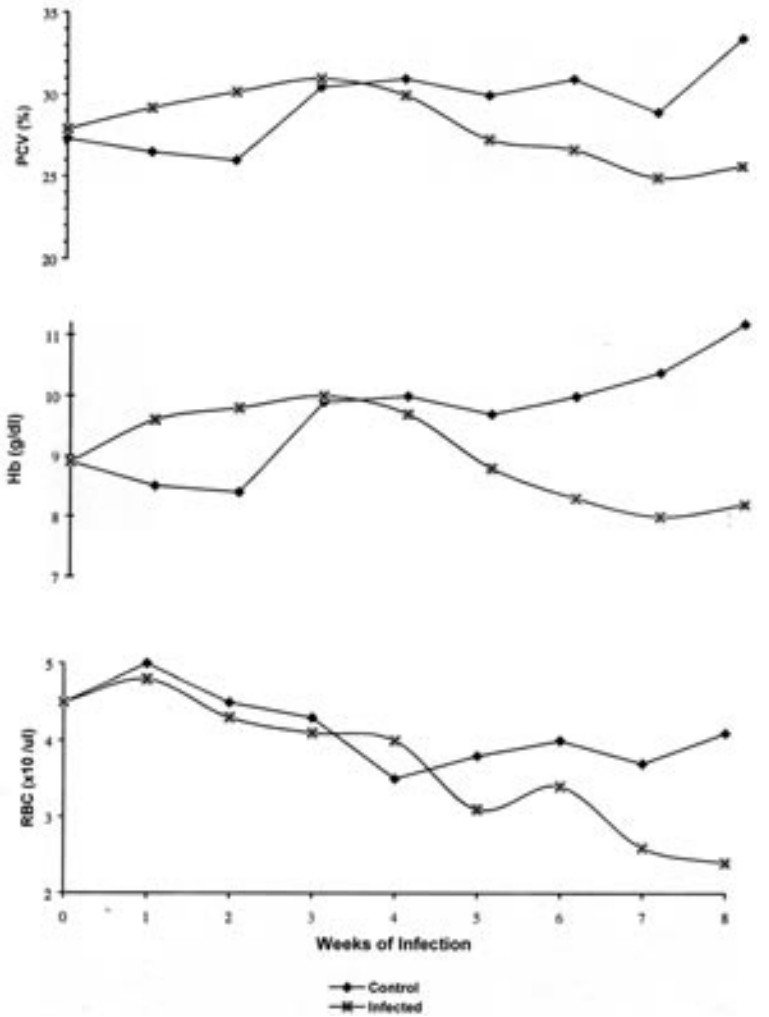

Fig. 1. Sequential changes in the mean PCV, Hb and RBC values of control and infected dogs

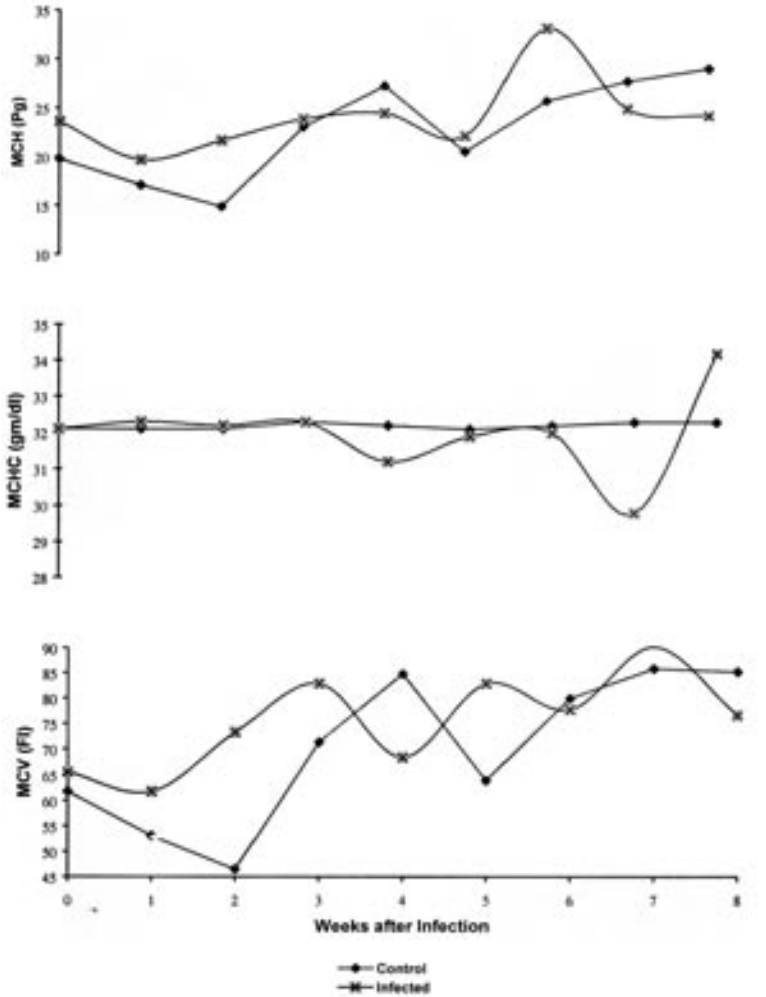

Fig. 2. Sequential changes in the MCH, MCHC and MCV values of infected and control dogs

Table 1. Sequential changes in the mean erythrocyte values of control and *T. congolense*-infected dogs

	PCV (%)	Hb (g/dl)	RBC (x10^6/ml)	MCV (fl)	MCH (Pg)	MCHC (g/dl)
Week 0						
Control	27.5 ± 0.7	8.9 +0.9	4.7 ± 0.4	61.7 ± 9.3	19.8 ± 2.9	32.1 ± 0.07
Infected	27.7 ± 0.5	8.9 ± 0.1	4.45 ± 0.6	65.1 ± 0.9	23.6 ± 5.9	32.1 ± 0.1
Week 2						
Control	26.0 ± 2.8	8.4 ± 0.9	5.6 ± 0.1	46.5 ± 6.2	14.9 ± 1.9	32.1 ± 0.1
Infected	30.2 ± 5.3	9.8 ± 1.7	4.3 ± 0.8	73.3 ± 26.1	21.7 ± 8.71	32.2 ± 0.12
Week 4						
Control	31.0 ± 2.8	10.0 ± 1.0	3.5 ± 0.6	84.9 ± 17.3	27.3 ± 5.8	32.2 ± 0.21
Infected	30.0 ± 1.2	9.7 ± 0.4	4.0 ± 1.1	68.4 ± 18.7	24.5 ± 4.9	31.2 ± 2.1
Week 6						
Control	31.0 ± 5.6	10.1 ± 1.8	4.0 ± 1.4	80.0 ± 14.4	25.8 ± 4.5	32.2 ± 0.07
Infected	26.7 ± 1.15	8.3 ± 0.4	3.4 ± 0.42	77.8 ± 8.2	33.2 ± 0.1	32.0 ± 0.14
Week 8						
Control	33.5 ± 0.71	11.2 ± 0.2	4.1 ± 0.2	85.3 ± 6.2	29.1 ± 0.14	32.3 ± 0.07
Infected	25.6 ± 3.8	8.2 ± 1.3	2.4 ± 1.0	76.7 ± 11.5	24.3 ± 6.7	34.2 ± 3.7

a sporadic maximum decrease to a value of 29.8 ± 2.3 gm/dl by week 7, and thereafter an increase of 34.2 ± 3.7 gm/dl ($P>0.05$) by week eight. The MCV values of the control dogs decreased in the first two weeks but thereafter showed a fluctuating increase throughout the observation period, while those of the MCH and MCHC fluctuated within normal ranges (Fig. 2).

Discussion

Trypanosoma congolense-infected puppies did not become anaemic until after four weeks of infection. This was characterized by a drop in mean PCV, Hb and RBC counts, which was mild in nature. These observations differed from the acute disease arising from *T. congolense*-infected Sahelian goats (N'DOUTAMIA et al., 2002) which became lethal within four weeks and was characterized by sharp drop in the PCV, reaching the 15% critical point at which the animals died, while some were unable to walk. Similarly, BENGALY et al. (2002) reported severe disease arising from savannah-type *T. congolense* infection

in cattle that was accompanied by marked drop in PCV and leucocyte counts, resulting in death between 29 and 59 days post-infection. There is little information on any work comparing the trypanosusceptibility status of different breeds of dog in Nigeria and West Africa (WIEGERS, 1996). Although different genetically distinct types of *T. congolense* also differed in their pathogenicity (BENGALY et al., 2002), the ability of the seven-week-old dogs to resist anaemia for four weeks and show only a mild drop in the PCV and Hb thereafter, was indicative of trypanotolence in the local breed of dog (D'IETEREN et al., 1998; MOLOO et al., 1999).

DARGIE et al. (1979) earlier reported more severe anaemia in zebu than in trypanotolerant N'dama cattle infected with *T. congolense*. Erythrocyte changes in the *T. congolense*-infected dogs were also characterized by general macrocytosis which was observable from the second week of infection, but was higher in the last four weeks of infection, corresponding to the period of mild anaemia. Macrocytosis in African trypanosomosis usually arises from the circulation of large numbers of reticulocytes which characterizes responsive anaemia known to occur during the acute phase of the anaemia (ANOSA, 1988; IGBOKWE and ANOSA, 1989; ABENGA, 1997). The increase in MCV which was also observable in the control dogs suggests that these increases were also due to artificial anaemia as a result of weekly bleeding of the young dogs.

MCH, as well as MCHC, values of the dogs also did not differ significantly from those of control signifying normochromasia (ANOSA, 1988). Slight and sporadic increases in the values of MCH were probably due to free circulating haemoglobin arising from hemolysis of RBC (IGBOKWE, 1986). The sporadic decrease in MCHC at week 7 may be due to increased circulation of reticulocytes due to over bleeding of the young puppies. MCHC was reported to be normal in *T. congolense* infected cattle (VALLI et al., 1978), while it was depressed in *T. brucei* infection of rabbits (JENKINS et al., 1980) and in older calves infected with *T. congolense* (VALLI and MILLS, 1980). MCH was also unaltered in *T. brucei*-infected rabbits (JENKINS et al., 1980), but increased in *T. congolense*-infected calves (VALLI and MILLS, 1980). Macrocytic normochromic anaemia observed in the *T. congolense*-infected young dogs suggests that the infection had little adverse effect on erythropoiesis and iron incorporation into red cell precursors (DARGIE et al., 1979). Macrocytic normochromic anaemia was reported in the early acute phase of *T. vivax* infection of cattle (ANOSA, 1988).

Factors involved in the pathogenesis of anaemia in African trypanosomosis include haemolysis, haemodilution, haemorrhages and dyshaematopoiesis (ANOSA, 1988). Mild anaemia observed in the infected dogs points to the low antigenic nature of the strain of T.congolense used. This may have resulted in low antigenic activation of macrophages involved in the erythrophagocytosis (ANOSA et al., 1997) and other factors leading to the development of anaemia in the infected puppies.

Conclusion

Trypanosoma congolense caused mild anaemia which was delayed until the second half of the observation period, signifying the tolerance of the young local dogs to the infection. However, the infected dogs did not attain full erythrocyte values as those of control, confirming that anaemia is an important factor associated with retarded growth in young animals infected with African trypanosomosis.

Acknowledgements

The authors wish to thank Mr. William Okolofo and Markus Maikaje for their technical assistance, as well as the Director General of the Nigerian Institute for Trypanosomosis Research, Kaduna, Nigeria, for granting permission to publish.

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Received: 22 March 2004
Accepted: 1 March 2005

ABENGA, J. N., C. O. EZEBUIRO, K. DAVID, A. O. FAJINMI, S. SAMDI: Istraživanje anemije u nigerijske domaće štenadi invadirane vrstom *Trypanosoma congolense*. Vet. arhiv 75, 165-174, 2005.

SAŽETAK

Utjecaj invazije vrstom *Trypanosoma congolense* na hematološke pokazatelje u nigerijskih domaćih pasa istražen je na 6 štenadi invadirane s 10^6 parazita. Invazija se očitovala blagom anemijom s laganim padom vrijednosti hematokrita, hemoglobina i broja eritrocita, ali tek u drugoj polovini 8-tjednog promatranja. Anemija je bila makrocitna, normokromnog tipa. Blagi pad ukupnih vrijednosti eritrocita u invadirane štenadi može se pripisati toleranciji domaćih pasa za *T. congolense*. Međutim, vrijednosti eritrocita u invadirane štenadi nisu dostigle one u kontrolne skupine, što znači da slične promjene u invadiranih mladih životinja mogu doprinijeti njihovu usporenom razvoju zbog invazije tripanosomama.

Ključne riječi: anemija, pas, *Trypanosoma congolense*, tolerancija na tripanosome