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Effects on haematological parameters and pathology of internal organs of *Trypanosoma brucei brucei* infected albino rats

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Abstract
The effects of *Trypanosoma brucei brucei* on the haematological parameters and pathology of internal organs of albino rats were studied in Michael Okpara University of Agriculture, Umudike to corroborate the reports or otherwise of some other researchers as is allowed in science. Three groups of albino rats (A-C), with 3rats in each group were used. Group A served as the control (uninfected). There was a significant increase ($p<0.05$) in the total white blood cell counts (WBC) (7.49-15.70) and significant decreases ($p<0.05$) in the red blood cell counts (RBC) (6.13-2.62), packed cell volume (PCV) (36.6-9.7%), and haemoglobin concentration (Hb) (14.69-4.05g/dl) of all the infected rats, an indication that anaemia had set in. The gross pathological effects on the internal organs showed significant enlargement of the spleen (splenomegaly) and slight enlargement of the liver (hepatomegaly). The heart and liver appeared anaemic. This work has shown that *Trypanosoma brucei brucei* is pathogenic in albino rats thus corroborating the work of some other researchers.

Key words: Trypanosoma, albino rat, haematology, pathology and blood characteristics
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Introduction

Trypanosomiasis is a clinical disease caused by the protozoa *Trypanosoma* and transmitted by the tse- tse fly (*Glossina species*). The incidence of trypanosomiasis has been of great concern in the tropics. The trypanosomes which affect both man and animals have been subdivided into two, namely the haematic groups(*Trypanosoma congolense and T. vivax*) which always remain in the plasma of the host's blood and the tissue invading group (*T.brucei, T.evansi, T.rhodesiense, T.gambiense and T. equiperdum*) which are found extravascularly and intravascularly (Anosa *et a*l; 1977).

Over 66million women, men and children in 36 countries of sub-Sahara Africa suffer from Human African Trypanosomiasis (HAT). If untreated, it gives no respite from suffering and ultimately ends in death. (Microbiologybytes, 2007).The main species of importance in livestock are *T.vivax, T. congolense* and to a lesser extent *T. brucei* causing Animal African Trypanosomiasis (AAT). AAT constitutes a menace leading to severe reduction in productivity, loss of weight, milk yield, reduction in carcass quality and capacity for work due to the failure of livestock to utilize available food as efficiently as healthy animals (Itard, 1989; Abenga *et al*; 2002; The Merck Vet Manual, 2003). This work set out to study the haematological changes in infected albino rats, observe the pathological effects on the morphology of some organs of the *Trypanosoma brucei brucei* infected albino rats and compare with those of the uninfected rats.

Materials and Methods

Study area: The study was conducted in Michael Okpara University of Agriculture, Umudike in Ikwuano L.G.A. Abia State. (Longitude $7\ ^{0}33^{l}$ E and latitude $5\ ^{0}29^{1}$ N.)

Data Collection: The rats used for this work were inbred strains of laboratory white rats *Rattus rattus novergicus* collected from the Veterinary Parasitology Department of University of Nigeria, Nsukka. The *Trypanosoma brucei brucei* that were used were collected from infected rats in the Veterinary Pathology Department of University of Nigeria, Nsukka. These were maintained in vivo by passaging into four uninfected adult albino rats whose weights ranged between 260-275g in the Department of Biological Sciences laboratory of Michael Okpara University of Agriculture Umudike (MOUAU) before being used for the study. These served as the reservoir for the parasites used for this work.

Experimental Layout: Twelve healthy albino rats of both sexes weighing between 260-275g were used for the study. They were housed in clean cages at room temperature in the animal house of the Department of Biological Sciences, MOUAU. All animals had free access to drinking water which was given ad libitum and were fed on commercial diets (vital feeds). They were allowed to acclimatize to their environment and diet for seven days before the experiment commenced. The rats were all screened for the presence of blood parasites using wet and Giemsa stained thin films (Cheesbrough, 1999; Taylor, 2008). The rats were grouped into three (A-C) of three rats each to make provision for replicates. Groups B and C were infected with *Trypanosoma brucei brucei* (approximately $1x10^6$ trypanosomes) while group A served as the control. The remaining three were kept as reserve in case of emergency.

The parasitaemia level was monitored in the course of the work till the end of the experiment. The packed cell volume (PCV), white blood cells counts (WBC), red blood cells (RBC) counts and haemoglobin (Hb) concentration of each rat in the various groups were determined before inoculation with the *Trypanosoma brucei brucei* and subsequently on four days interval till the end of the experiment. A rat from each group was sacrificed on eight days interval to note the pathological effects of the infection on the rats' organs. The experiment was terminated on the 28^{th} day post-infection. The mean PCV values, RBC, WBC counts and Hb values of each group of rats were recorded (Losos *et al;* 1979).

Blood Collection and Inoculations: Blood for inoculation was collected from the tails of infected rats in which the trypanosomes were passaged into sterile bottles with anticoagulant EDTA. For easy flow of blood, the tail was massaged, cleaned with methylated spirit soaked cotton wool and then pricked with a sterile lancet before the collection of blood. Inoculum used was 0.5ml of blood in normal saline containing approximately $1x10^6$ trypanosomes. This was used to challenge the experimental rats intraperitoneally.

Determination of Haematological Parameters: The RBC and the WBC counts were determined using the new improved Neubauer counting chamber while the PCV (%) was determined using the haematocrit centrifugation technique (HCT) (Baker, 1985; Dacie &Lewis, 1994; Cheesbrough, 2005). Hb(g/dl) value was estimated using the cyanmethaemoglobin method with spectrophotometer (Dacie& Lewis,1994; Cheesbrough, 2004).

Pathological Studies: One rat from each group (A, B, C) was sacrificed on days 8 and 16 post inoculation after the haematological tests had been done for the pathological studies. On day 24, the last rat in group B died while that of group C died on day 27. The last rat in the experiment from group A (control) was sacrificed on day 28 post inoculation. Data was analysed using SPSS Version 16.0 (2007)

Results

The infected rats from groups B and C had decreases in RBC counts, PCV and Hb values, an indication that anaemia had set in. However there was a progressive increase in the WBC counts as the infection progressed. Pathological changes were observed in the organs (spleen, liver, heart, and kidney) examined in the infected rats. In group B (infected), the mean PCV value dropped from 37.6% on day 0 pre inoculation to 13.2% on day 24 post inoculation. In group C(infected) the mean PCV decreased from 36.6% on day 0 pre inoculation to 9.7% on day 24 post inoculation while in group A (control), there was an insignificant increase in the mean PCV values from 38.6% on day 0 pre inoculation to 39.3% on day 28 post inoculation (Table 1).

Similarly, the mean RBC counts decreased from 6.13 on day 0 to 3.37 on day 20 post inoculation in group B while in group C, the mean RBC counts decreased from 6.16 on day 0 pre inoculation to 2.62 on day 24 post inoculation. There was an insignificant variation in the RBC counts in group A from 6.17 on day 0 to 6.12 on day 28 post inoculation (Table 2).

The Hb concentration decreased from 14.69 on day 0 to 5.80g/dl on day 20 post inoculation in group B and the last rat died on day 24 post inoculation while in group C, the Hb concentration decreased from 14.97 on day 0 to 4.05g/dl on day 24 post inoculation. The last rat died on day 27 post inoculation. In group A, there was slight variation in the mean Hb values from 14.73 on day 0 to 14.80g/dl on day 28 post inoculation (Table 3).

On the contrary however, the mean WBC counts increased from 7.49 on day 0 to 12.75 on day 20 post inoculation in group B and from 7.60 on day 0 to 15.70 on day 24 post inoculation in group C, while in group A it varied slightly from 6.82 on day 0 to 6.87 on day 28 post inoculation (Table 4).

The gross pathological changes were indicated by the enlargements of the spleens (splenomegaly), the livers (hepatomegaly), the hearts of all the infected rats and the anaemic appearances of the spleens and the kidneys. The spleens of the infected rats were about 3 times the size of those of the control animals. The hearts and kidneys of the infected rats showed slight increases in size (Table 5).

Discussion

Each infected rat received the same amount of infective trypanosome inoculum to ensure the elimination of possible influence of the infective dose on the pre-patent period and subsequent parasitaemia (Murray and Dexter, 1988). The infected rats showed high parasitaemia and gross pathological changes on their observed internal organs, an observation which is supported by the report that *T.brucei brucei* is highly pathogenic to rats (Losos and Ikede, 1972).

The results of this work indicate a significant reduction in the mean values($p<0.05$) of the PCV, RBCs count, and Hb estimations due to the presence of the *Trypanosoma brucei brucei.* The decrease in these haematological values suggests anaemia (Jenkins *et al* 1980; Abenga *et al;* 2002) following ingestion of blood by the parasites (Losos and Ikede, 1972).The increase in WBCs count in the infected rats is an indication of infection and could be attributed to the body employing its immune arsenals to fight the invading parasites and in the process of immune response enhance the production of more WBCs. (McCorrie *et al;* 1980, Mare, 2000). Very slight and insignificant variations were seen in the PCV, RBCs WBCs counts and Hb estimations of the rats in the control group.

Loss of appetite and a marked weakness were noticed in the infected rats on day 20 post inoculation. The rats were dull and not as agile as those in the control group. This continued with some discharges from the eyes up to the 27th day post inoculation when the last infected rat died. All these are pointers to the pathogenic effects of *T. brucei brucei* and the setting in of the deadly disease Trypanosomiasis. *T.brucei brucei* is tissue invading. This could be the reason it is highly pathogenic (Anosa, 1977).

Table 1: Packed cell volume (PCV)(%) values of *T. brucei brucei* infected and uninfected albino rats

GROUPS		RATS	DAYS OF EXPERIMENT							
			0	4	8	12	16	20	24	28
Group	A	1	38.8	37.0	37.6	S	-	-	-	-
Uninfected		2	37.9	38.6	38.0	38.6	37.5	S	-	-
(Control)		3	39.0	37.4	39.4	35.9	39.4	37.7	39.0	38.9
MEAN			38.6	37.6	38.3	37.4	38.5	37.7	39.0	38.9
Group	B	1	38.6	34.1	31.3	S	-	-	-	-
(infected)		2	36.8	31.0	22.5	20.1	14.5	S	-	-
		3	37.4	33.3	23.1	25.2	18.5	13.2	D	-
MEAN			37.6	32.8	25.6	22.6	16.5	13.2	D	-
Group	C	1	38.3	39.1	30.5	S	-	-	-	-
(infected)		2	34.8	30.9	25.8	22.8	17.4	S	-	-
		3	36.6	34.3	24.4	26.2	16.7	13.1	9.7	D
MEAN			36.6	34.58	26.9	24.5	17.05	13.1	9.7	D

Key: S = Sacrificed, D = Dead, - = Nil

The invasion of tissues by these parasites probably resulted in the marked effects (splenomegaly and hepatomegaly) on the internal organs of the infected rats. These go to further buttress other reports that *T.brucei brucei* has adverse effects on the general health of rats (Losos, 1979). Going by the results obtained in this work, *T.brucei brucei* have been seen to have adverse pathogenic effects on the haematological parameters and pathology of the internal organs of the infected albino rats, thus corroborating earlier works that have been done. The work has also shown that albino rats are susceptible to infection with *Trypanosoma brucei brucei* and not trypanotolerant as in the West African short horned cattle. Similar effects could also be exerted on larger animals which serve as sources of food (protein) and income to man. Effective surveillance, control and treatment measures are advocated to protect humans and animals from infection especially animals that are not trypanotolerant.

Table 2: Red blood cells (RBC) 10^{12}/L counts of *T. brucei brucei* infected and uninfected albino rats

GROUPS		RATS	DAYS OF EXPERIMENT							
			0	4	8	12	16	20	24	28
Group	A	1	6.48	6.00	6.18	6.18	S	-	-	-
Uninfected		2	6.37	5.96	5.86	5.86	5.85	S	-	-
(Control)		3	5.67	6.26	5.76	5.76	5.88	6.10	6.05	6.12
MEAN			6.17	6.07	5.84	5.84	5.86	6.10	6.05	6.12
Group	B	1	6.45	6.01	5.60	5.60	S	-	-	-
(infected)		2	5.97	5.16	4.83	4.83	3.38	S	-	-
		3	5.98	5.81	5.90	4.86	4.10	D	D	D
MEAN			6.13	5.66	5.43	4.84	3.74	3.37	D	D
Group	C	1	6.84	6.37	5.94	S	-	-	-	-
(infected)		2	6.15	5.71	5.26	5.04	5.04	S	-	-
		3	5.50	5.05	4.71	4.28	3.91	3.22	2.62	D
MEAN			6.16	5.71	5.30	4.66	4.09	3.22	2.62	D

Key: S = Sacrificed, D = Dead, - = Nil

Table 3: Haemoglobin (Hb) (g/dl) values of *T. brucei brucei* infected and uninfected albino rats

GROUPS		RATS	DAYS OF EXPERIMENT							
			0	4	8	12	16	20	24	28
Group	A	1	14.80	14.70	14.58	S	-	-	-	-
Uninfected		2	14.75	14.62	14.68	14.85	14.78	S	-	-
(Control)		3	14.68	14.60	14.78	14.90	14.70	14.82	14.78	14.80
MEAN			14.73	14.64	14.67	14.87	14.74	14.82	14.78	14.80
Group	B	1	14.78	13.20	10.20	S	-	-	-	-
(infected)		2	14.70	12.90	12.00	10.00	8.60	S	-	-
		3	14.60	14.20	11.10	7.00	6.80	5.80	D	-
MEAN			14.69	13.40	11.20	8.50	7.70	5.80	D	-
Group	C	1	14.90	14.00	12.10	S	-	-	-	-
(infected)		2	14.60	13.30	10.20	9.70	7.14	S	-	-
		3	15.30	14.20	11.09	9.35	6.83	5.34	4.05	D
MEAN			14.97	13.83	11.13	9.52	6.96	5.34	4.05	D

Key: S = Sacrificed, D = Dead, - = Nil

Table 4: White Blood Cells (WBC) counts (10^9/L) of *T. brucei brucei* infected and uninfected albino rats

GROUPS	RATS	DAYS OF EXPERIMENT							
		0	4	8	12	16	20	24	28
Group A	1	7.38	6.86	6.40	S	-	-	-	-
Uninfected	2	6.47	7.55	7.05	6.60	6.14	S	-	-
(Control)	3	6.61	6.48	6.68	6.50	6.82	6.72	6.21	6.37
MEAN		6.82	6.96	6.71	6.55	6.46	6.72	6.21	6.37
Group B	1	7.54	7.87	8.74	S	-	-	-	-
(infected)	2	6.78	8.84	9.19	9.23	10.06	S	-	-
	3	8.17	8.20	8.91	10.20	11.56	12.75	D	_
MEAN		7.49	8.03	8.94	9.72	10.79	12.75	D	-
Group C	1	6.97	9.03	9.38	S	-	-	-	-
(infected)	2	7.89	8.13	9.09	9.56	10.21	S	-	-
	3	7.94	7.97	8.68	9.97	11.29	12.52	15.70	D
MEAN		7.60	8.37	9.05	9.76	10.75	12.52	15.70	D

Key: S = Sacrificed, D = Dead, - = Nil

Table 5: Summary of the changes in the weight of organs (g) in *T. brucei brucei* infected and uninfected albino rats

Groups	Organs	8	16	24	28
Group	Liver	8.0	7.9	-	8.1
Uninfected	Spleen	0.9	1.0	-	1.0
	Kidney	1.0	0.9	-	1.1
	Heart	0.9	0.9	-	1.0
Group	Liver	8.7	8.5	D	-
(infected)	Spleen	3.3	3.7	D	-
	Kidney	1.2	1.2	D	-
	Heart	1.0	1.1	D	-
Group	Liver	8.5	8.8	9.0	D
(infected)	Spleen	3.1	3.6	4.0	D
	Kidney	1.1	1.1	1.2	D
	Heart	1.0	1.1	1.1	D

Key: D = Dead, - = Nil

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