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A comparative study on the susceptibility of male and female albino mice to *Trypanosoma brucei brucei*

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Background & objectives: Trypanosomiasis has remained a major set-back in the development of livestock farming in tropical Africa. Thus the need for ascertaining the trypanotolerant levels of domestic animal breeds and possible improvement on them cannot be over-emphasised.

Methods: Level of trypanotolerance in animals was compared between sexes using albino mice infected with a Nigerian strain of *Trypanosoma brucei brucei* at a 50% mouse lethal dose (MLD_{50}).

Results: The male mice showed unrestrained parasite growth with a prepatent period (PP) of two days and a mean survival period (MSP) of six days corresponding to a gradual decrease in packed cell volume (PCV), body weight, diet response and white blood cells (WBC) count to the time of death. Their female counterparts showed a PP of three days and MSP of ten days with a similar PCV gradient but a refractory WBC count. There was no significant difference in the differential leucocytes count in both sexes. However, the eosinophils count was significantly higher in the infected animals. It was found that female albino mice exercised more parasite restraint than their male counterparts.

Interpretation & conclusion: The result suggests that the female animals may be more trypanotolerant hence may be more useful in protein production in trypanosomiasis endemic areas. However, further research using large domestic breeds like goats and sheep may be required to confirm the hypothesis.

Key words Albino mice – comparative study – endemicity – sex – trypanotolerance

Trypanosomiasis has been a major constraint to agricultural development particularly in livestock production in many countries along the equatorial region of sub-Saharan Africa affecting two third of the land in Nigeria[1–3].

The disease results in extensive pathological and economic damage with more impact at herd level ranging from depressing all aspects of protein production to reducing herd size[4–6]. Various intervention methods have been applied but have proved inadequate due to limitations[7,8]. Several rural developers need considerable resources and strategies for the disease control. The resources are most often inadequate or lacking with the attendant, widespread of the disease and the high level of malnutrition in the communities.

Although few cattle are trypanotolerant and can be bred in trypanosomiasis endemic regions, they are expensive exotic breed that are not affordable by the average rural farmer. If the threat of trypanosomiasis is to be removed from these regions or reduced for the

production of ample protein, more research on management strategies involving the rural farmers should be encouraged.

Ascertaining the level of trypanotolerance in indigenous cattle for breeding as supplement and/or alternative to trypanotolerant cattle may be rewarding. Those with higher level of trypanotolerance could be used on a larger scale for breeding as alternative to the exotic breed. The research on sex related trypanotolerance is designed to study the differences in the level of parasite restraint between male and female albino mice as a base for further research that will encourage the breeding more of the indigenous trypanotolerant animals in endemic regions for protein production.

Material & Methods

The study was carried out in the Parasitology Division of the National Institute for Trypanosomiasis Research (NITR), Vom, Plateau State, Nigeria. The albino mice and the *Trypanosoma brucei brucei* species were obtained from NITR, Vom. The *T. brucei brucei* used was an isolated strain from a cow in Gboko in Benue State, Nigeria, that has been attenuated and cryopreserved in liquid Nitrogen at – 196°C. This was reactivated and maintained in clean albino mice few days before the commencement of the research.

A total of 120 adult albino mice grouped into A, B and C with equal number of males and females (20 each) in separately labeled cages were individually numbered and preconditioned on commercial growers mash for a period of seven days. An appropriate inoculum's dose of the *T. brucei brucei* from the donor mice was determined by Reed and Muench as explained by Hoskins[9] and used to infect mice in group A. The mice in groups A and B were subjected to daily ocular blood collection for the estimation of PCV, WBC and differential leucocytes count by Bain's method[10] and the parasite load by the rapid matching and limited counting method of Herbert and Lumsden[11]. Mean levels of all the parameters were recorded daily.

Mice in groups B and C were not infected and the latter were not subjected to the traumatic ocular blood collection method. Whilst group B served to eliminate the effect of the traumatic blood collection process as a possible cause of death, and to obtain normal blood parameters, group C served to monitor body weight and feeding response to the commercial growers mash feed.

Inoculum's dose

The appropriate inoculum's dose was estimated from the titre obtained from the proportion of the mice observed dead after inoculation with a series of the consecutive various trypanosomes' dilutions. Ten mice per dilution were intraperitoneally inoculated with 0.02 ml of the dilution.

The proportion of mice dead was summed up cumulatively and the total infected was expressed as a percentage. The inoculum's dose used in the experiment was prepared by making 10^{-4} dilution of the blood of the donor mice from which 6.3 ml was further diluted with 3.7 ml of sterile phosphate buffered saline.

Statistical analysis

The student *t*-test[12] was used to test the significance of the differences in trypanotolerance between the male and female albino mice. Values for $p < 0.05$ were considered statistically significant.

Results

Group B mice survived the traumatic blood collection process and remained alive together with those in group C to the end of the experiment. Mean PCV values of 45.7 and 46.1% and WBC counts (10^3cells/ml) of 5.4 and 6.1 for males and females respectively were obtained for group B mice. This group remained clean of parasitic infection with normal body weights, diet response and normal differential leucocyte counts throughout the course of the experiment (Table 1). Group C mice also remained active with normal body

Table 1. Values (mean ± SD) of packed cell volume (PCV), white blood cells (WBC), body weight and diet response (GM g/day) in control mice (Group B)

MSP (Days)	PCV(%)		WBC (10^3cells/ml)		Body weight (g)		GM g/day	
	Male	Female	Male	Female	Male	Female	Male	Female
1	45 ± 0.6	46 ± 1.0	5 ± 1.0	5 ± 1.0	45 ± 1.1	41 ± 0.2	4 ± 0.3	5 ± 0.1
2	46 ± 1.1	45 ± 1.5	6 ± 0.1	7 ± 0.9	46 ± 0.1	41 ± 1.5	4 ± 0.4	4 ± 0.1
3	46 ± 0.8	46 ± 0.5	5 ± 1.2	6 ± 0.5	44 ± 1.5	41 ± 2.1	5 ± 0.4	5 ± 0.1
4	46 ± 0.9	47 ± 0.3	5 ± 1.5	6 ± 0.4	44 ± 2.0	39 ± 2.0	5 ± 0.3	5 ± 0.2
5	45 ± 1.2	46 ± 0.5	6 ± 0.9	7 ± 1.2	46 ± 0.1	40 ± 0.4	5 ± 0.4	5 ± 0.3
6	46 ± 0.7	46 ± 0.2	6 ± 0.5	6 ± 0.2	45 ± 0.2	41 ± 2.0	6 ± 0.5	5 ± 0.6
7	46 ± 0.5	46 ± 1.2	5 ± 1.3	6 ± 0.4	45 ± 0.4	40 ± 1.0	5 ± 0.2	5 ± 0.4
8	46 ± 1.0	47 ± 0.4	6 ± 0.8	6 ± 0.5	44 ± 2.1	40 ± 0.1	5 ± 0.4	6 ± 0.4
9	46 ± 0.5	46 ± 0.6	5 ± 0.9	6 ± 1.0	44 ± 2.2	39 ± 1.5	5 ± 0.5	6 ± 0.3
10	45 ± 1.8	46 ± 0.9	5 ± 1.4	6 ± 0.9	46 ± 0.1	39 ± 2.0	4 ± 0.4	5 ± 0.4
	[t = 1.7 (df = 18) p >0.05] CL = 0.4 ± 0.5		[t = 2.9 (df = 18) p <0.05] CL = 0.7 ± 0.5		[t = 15.3 (df = 18) p < 0.05] CL = 4.9 ± 0.7		[t =1.1 (df=18) p >0.5] CL = 0.3 ± 0.6	

CL—Confidence limit; p—Probability; df—Degree of freedom; t—Students *t*-test (Calculated); MSP—Mean survival period; GM—Growers mash in grams consumed by a mouse/day.

weight and good feeding response to the end of the experiment (Table 2).

The initial mean body weights of group A mice on infection were 45 and 40 g for male and female respectively and their respective weights and diet response to the time of death were reduced significantly ($p < 0.05$) (Table 3). Similarly, their initial PCV values were 45% for males and 46% for the females but reduced to 28 and 29% on the sixth day post-infection. Mean white blood cell counts were also reduced to 2 in males and 4 in females on the sixth day but was raised to 5 on the eighth and ninth days in the females. The rate of fall in PCV in both infected male and female albino mice was the same ($p > 0.05$), but that of WBC count differed significantly ($p < 0.05$). The differential leucocyte counts were raised both in males and females with higher increases in the values of eosinophils. Similarly, the pattern of parasite growth in both sexes was linear with corresponding reduction in

Table 2. Values (mean ± SD) of body weight and diet response (GM g/day) of control mice in group C

MSP (Days)	Body weight (g)		GM g/day	
	Male	Female	Male	Female
1	45 ± 1.0	40 ± 0.1	5 ± 0.3	5 ± 0.4
2	44 ± 2.0	39 ± 1.0	5 ± 0.1	5 ± 0.4
3	44 ± 2.0	41 ± 0.5	5 ± 0.1	5 ± 0.2
4	45 ± 1.0	42 ± 1.4	6 ± 0.3	5 ± 0.3
5	45 ± 1.3	43 ± 1.5	6 ± 0.4	5 ± 0.1
6	46 ± 1.0	44 ± 2.0	5 ± 0.3	6 ± 0.1
7	46 ± 2.0	44 ± 1.0	5 ± 0.4	6 ± 0.2
8	45 ± 2.0	44 ± 1.0	5 ± 0.3	6 ± 0.1
9	45 ± 2.0	43 ± 0.5	5 ± 0.2	5 ± 0.3
10	45 ± 1.0	43 ± 1.0	5 ± 0.2	5 ± 0.4
	[t = 0.5 (df = 18) p>0.05] CL = 0.1 ±1.4		[t = 5.5 (df = 18) p<0.05] CL = 3.3 ± 1.3	

Table 3. Values (mean ± SD) of packed cell volume (PCV), total white blood cells (WBC), differential white blood cells counts, parasite level, percentage mortality, body weight and diet response (GM g/day) in infected mice (Group A)

MSP (Days)	PCV (%)		WBC (x10^3 cells/ml)		Parasite load (x10^3 cells/ml)		Mortality (%)		Body weight (g)		GM g/day	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
1	45 ± 1.0	46 ± 0.3	5 ± 0.5	6 ± 0.9	0	0	0	0	45 ± 0.1	40 ± 0.2	5 ± 0.1	4 ± 0.9
2	44 ± 0.5	45 ± 0.9	5 ± 0.8	5 ± 0.4	1	0.1	0	0	46 ± 1.0	42 ± 3.0	5 ± 0.4	4 ± 0.8
3	42 ± 0.1	43 ± 0.4	4 ± 0.9	5 ± 0.9	6.5	1	10	0	44 ± 3.0	41 ± 3.0	4 ± 0.2	5 ± 0.3
4	40 ± 0.4	39 ± 0.6	4 ± 0.3	4 ± 0.6	260	6	30	0	40 ± 2.0	40 ± 3.0	3 ± 0.2	5 ± 0.3
5	36 ± 1.2	31 ± 1.2	3 ± 0.6	5 ± 1.0	1800	120	70	20	33 ± 2.0	38 ± 2.0	3 ± 0.6	4 ± 0.2
6	28 ± 0.5	29 ± 1.0	2 ± 1.1	4 ± 0.6	$\geq$ 5000	2500	90	35	28 ± 3.0	38 ± 2.0	2 ± 0.4	4 ± 0.2
7	0	21 ± 0.9	0	4 ± 0.8	0	35000	100	60	0	36 ± 2.0	0	2 ± 0.5
8	0	20 ± 0.6	0	5 ± 0.5	0	Massive	0	70	0	32 ± 2.0	0	1 ± 0.2
9	0	20 ± 0.8	0	5 ± 1.0	0	Massive	0	90	0	28 ± 1.0	0	0
10	0	14 ± 0.6	0	4 ± 0.9	0	Massive	0	100	0	26 ± 2.0	0	0
	[t = 1.0 (df = 18) p >0.05] CL = 7.2 ± 1.6		[t = 3.4 (df = 18) p<0.05] CL = 2.4 ± 1.5						[t = 25.0 (df = 18) p<0.05] CL = 12.5 ± 1.1		[t = 0.8 (df = 18) p> 0.05] CL = 0.7 ±1.9	

Table 4. Values (Mean ± SD) of differential leucocytes count of groups A and B mice

Leucocytes (%)	Group A		Group B	
	Male	Female	Male	Female
Neutrophils	54.0 ± 2.0	56.0 ± 2.0	60.0 ± 2.0	60.0 ± 2.0
Eosinophils	10.0 ± 1.0	14.0 ± 1.0	2.0 ± 1.0	3.0 ± 2.0
Basophils	0	0	0	0
Lymphocytes	30.0 ± 1.0	40.0 ± 3.0	32.0 ± 1.0	33.0 ± 1.0
Monocytes	4.0 ± 2.0	5.0 ± 2.0	3.0 ± 1.0	4.0 ± 2.0
	[t = 0.01 (df = 8) p>0.05]		[t = 0.01 (df = 8) p>0.05]	

body weight, but the mean survival period (MSP) and parasite restraint differed (Table 4).

Discussion

Some differences in the relative trypanotolerance between male and female albino mice have been shown in this study from the leucocytes count, prepatent periods (PP), mean survival periods (MSP) and parasite restraint. Unlike PCV, diet response and body weight, which decreased with increase in parasite level in both sexes, total leucoctyes count remained almost the same or only slightly affected in the female mice but highly reduced in the male mice ($p < 0.05$). However, whilst other leucocytes diminished with increase in parasite load, eosinophils remained high till death, as they are the main defensive weapons of the immune system against parasitic infection.

The reduction in PCV may be due to the direct binding of the trypanosomes antigens with the specific receptors on the red blood cells giving rise to complexes eliciting the production of antibodies and endotoxins with the consequent lysing of the red blood cells leading to hypoxia, low metabolic rate, general weakness and death. This accounts for the shorter MSP of the mice and supporting earlier findings that *T. brucei brucei* establishes easily in laboratory animals with significant lowering of haematological indices[6,12].

The relationship between male and female mice susceptibility to infection with *T. brucei brucei* and the capacity to limit the level and duration of parasitaemia as well as to correlate with long time survival period has been studied[13,14]. Comparing the leucocytes count profiles over the period of infection, the significant decrease in male and the instant regeneration in females within the first week illustrate the polyclonal B-lymphoctye reactivation involved in the immune response and the level of tolerance by the female mice to *T. brucei brucei* infection. The study indicated that this factor of the B-lymphocytes is more active against trypanosomes in female albino mice than in male albino mice as shown in the level of lymphocytes proliferation in females during infection (Table 4). The PP, massive parasitaemia, mortality rate, MSP and the haematological parameters used in this study as indices for comparing trypanotolerance level between male and female albino mice may not be sufficient to draw inference but suggests that female albino mice are more trypanotolerant than their male counterparts.

It is the belief of the authors that similar results may be obtained when larger animals such as goats, sheeps, pigs and cattle are subjected to similar treatment. Based on this hypothesis, it is more likely that female animals are more trypanotolerant than their male counterparts more of their breeding in trypanosomiasis endemic regions for more protein and other agricultural products.

Trypanosomiasis control in countries like Nigeria, Gambia, Sierra Leone and other trypanosomes' endemic regions is imperative if adequate livestock products are to be produced. The breeding of exotic breeds is a very good venture, but this is often beyond the financial and managerial capabilities of the rural farmer.

Our findings in this report indicated that female animals are more trypanotolerant than their male counterparts, suggesting the breeding of more of the indigenous female than male trypanotolerant livestock as alternative to exotic breeds by rural farmers in trypanosomes' endemic countries.

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