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Experimental *Trypanosoma brucei* infection at immediate post partum period: effects on dam and the offspring

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ABSTRACT

Objective: To investigate the effects of immediate post-partum infection with *Trypanosoma brucei (T. brucei)* on dam and offspring. **Methods:** Sixty female Albino rats *(Rattus norvegicus)* weighing between 130-170 g were used as animal model. The animals were divided as follows: 25 infected between 1-5 days post partum; 10 infected unbred as positive controls; and 25 uninfected as negative controls. The following parameters were evaluated: packed cell volume (PCV), level of parasitaemia, survival time, litter size and litter weight at birth and on days 7, 14 and 21 post delivery, using conventional methods. Possible trans-mammary transmission of infection to litter through milk was also assessed. **Results:** The results showed a comparatively ($P< 0.05$) higher mean PCV value for the uninfected negative control on the 8th day post infection compared with the infected groups which corresponded with the increasing level of parasitaemia in the two infected groups. Mean litter size and litter weights were higher ($P< 0.05$) in the uninfected controls on the 21st day. Survival time in the infected groups were similar. No evidence of trans-mammary transfer of infection was recorded. **Conclusion:** *T. brucei* infection during immediate post partum period is detrimental to the dam and impairs growth of the offspring.

1. Introduction

Trypanosomosis is a complex disease of both animals and man, caused mainly by the pathogenic tsetse-transmitted protozoan haemoflagellate parasites belonging to the genus *Trypanosoma*[1,2]. The disease is endemic throughout sub-Saharan Africa and is a major constraint to livestock production[3]. The disease is considered, along with malaria, cancer and heart diseases, as among the most devastating to mankind[4]. The infection in animals is characterised by intermittent fever, anaemia, emaciation, impairment of immune function, and more importantly reproductive disorders. Death would occur if the animals remained untreated[5,6].

The endemic nature of the disease in sub-Saharan Africa makes it possible for female animals to be infected at any stage in life (pre-pubertal, pubertal, gestational and post partum periods). The parasite is classified into the "humoral" and "haematic" groups, depending on whether they invade tissues and organs of the host (humoral) or are confined to the blood vessels (haematic)[7]. One of the preferred predilections of the "humoral" subtype to which *Trypanosoma brucei (T. brucei)* belongs is the gonads where the infection is associated with adverse reproductive disorders such as irregular oestrous cycles, anoestrus, abortion, stillbirth and neonatal death[8-11].

Although reports on the clinical and pathological manifestations of trypanosomosis in domestic animals have been well documented[9,11-13], there is paucity of information on the effects of the parasite on the dam and offspring during the immediate post partum period.

The present study was therefore designed to determine the effects of immediate post partum T. brucei infection on the dam and offspring, including an evaluation of possible trans-mammary transfer of infection, using rat as model.

2. Materials and methods

2.1. Experimental animals

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Seventy two mature out-bred Sprague-Dawley albino rats (*Rattus norvegicus*) comprising 60 females and 12 males were used for the study. Just before commencing the study, all the animals weighed between 130 and 170 g, aged between 12 and 15 weeks. Throughout the study, they were housed at room temperature of 28-32 ℃ in the Laboratory Animal Unit of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka, using stainless steel cages. They were fed *ad libitum* with commercial feed (Top Feed Nigeria Ltd) containing 16% crude protein and 4.5% fibre. Clean water was also provided freely.

2.2. Source of trypanosomes

T. brucei stock maintained on albino rats was used for the study. This strain was originally isolated from a clinically infected dog and was identified at the Clinical Laboratory of the University of Nigeria Veterinary Teaching Hospital, Nsukka, Nigeria. Each infected animal received 2.50×10^5 trypanosomes administered intraperitoneally as 0.1 mL suspension in normal saline. Confirmation of infection was done starting from day 4 post-infection using haematocrit method as described by Murray *et al*[14].

2.3. Determination of successful mating

The vaginal plug method of Bennett & Vickery[15] modified by Ochiogu *et al*[16]. was used in determining successful mating. Briefly, each female rat sequentially paired with a male of proven fertility. The vaginal smear of the rat was made on a labelled clean glass slide with the aid of wet cotton swab dipped in fresh normal saline and inserted into the vagina to a depth of approximately 1 cm. The wet smear was examined grossly for the presence of protein coagulates (remnants of copulatory plug) which were taken as evidence of successful mating. This procedure was carried out at 12-hour intervals. The day remnants of the plug were found was regarded as day 1 of pregnancy. Increase in body weight was used in assessing progress of pregnancy.

2.4. Determination of packed cell volume (PCV) and level of parasitaemia

Packed cell volume (PCV) was determined by the microhaematocrit method[17], while the level of parasitaemia was assessed starting from day 4 post-infection and at intervals of 4 days using rapid matching method as described by Herbert and Lumsden[18]. Body temperature was monitored using a clinical thermometer.

2.5. Assessment for possible trans-mammary transmission of *T. brucei* infection

Blood collected from litters of infected mothers was screened and assessed for trypanosomes by the rapid matching method of Herbert and Lumsden[18] and the method of Murray *et al*[14].

2.6. Experimental design

Seventy two rats were used for the study. Twelve of the animals were males used solely for the purpose of mating. The remaining 60 females were randomly assigned to three groups as follows: Group 1 comprised 5 rats infected with trypanosomes on each of the 5 days post partum. Since data from this group were similar, the results of the 5 days post partum were merged. Group 2 comprised 25 rats that littered but not infected. This group served as negative control. Ten rats in group 3 were unbred but infected. This group served as the positive control. The following parameters were evaluated: PCV, level of parasitaemia, survival time (defined as the time, in days, between infection and death), and litter size and litter weight (g) at birth and on days 7, 14, and 21 post delivery. The pups of infected mothers were screened for trypanosomes during these periods to verify trans-mammary transmission of infection.

2.7. Data analysis

Data generated from the study were subjected to analysis of variance (ANOVA) and Student's *t*- test as appropriate. Variant means in the ANOVA tests were separated by the least significant difference (LSD) method. The results are presented as the means ± standard error of the means.

3. Results

3.1. Packed cell volume (PCV)

The results of PCV showed a significantly higher ($P < 0.05$) value before infection and on the 4th day post infection for the unbred but infected rats in group 3 than for rats in group 1 that were infected post partum and group 2 that littered but was uninfected (Table 1). However, from day 8 post infection until the 16th day, the mean PCV of the uninfected was significantly ($P<0.05$) higher than that of the infected groups.

Table 1

The effect of *T. brucei* infection on the PCV of rats infected within 5 days post partum, compared with uninfected and infected but unbred control groups.

Post infection period(days)	Mean packed cell volume (PCV) (%)		
	Infected within 5 days post partum (25)	Uninfected (25)	Infected unbred (10)
Before infection	$37.84^a \pm 0.37$	$38.02^a \pm 0.28$	$43.40^b \pm 0.42$
4	$38.68^a \pm 0.29$	$38.46^a \pm 0.36$	$43.30^b \pm 0.47$
8	$37.84^a \pm 0.34$	$40.36^b \pm 0.32$	$40.80^b \pm 0.53$
12	$36.72^a \pm 0.31$	$42.24^b \pm 0.33$	$38.90^a \pm 0.81$
16	$33.80^a \pm 0.44$	$43.37^b \pm 0.38$	$35.20^a \pm 0.64$

Results are presented as the means ± standard error of the means, numbers of animals used are indicated in parentheses, ^{ab}different superscripts in a row indicate significant difference between the means: $P < 0.05$.

3.2. Level of parasitaemia

There was no significant difference (P>0.05) between the mean levels of parasitaemia of the group infected post partum when compared with the infected but unbred control group (Table 2).

Table 2
The level of parasitaemia of rats infected with *T. brucei* within 5 days post partum compared with infected but unbred control group.

Post infection period (days)	Mean level of parasitaemia (10^6/mL)	
	Infected within 5 days post partum (25)	Infected but unbred control (10)
4	0.80 ± 0.22	0.60 ± 0.38
8	14.80 ± 4.20	74.16 ± 37.56
12	227.96 ± 32.45	344.79 ± 129.18
16	689.36 ± 55.08	656.00 ± 97.60

Results are presented as the means ± standard error of the means, numbers of animals used are indicated in parentheses.

3.3. Litter size and litter weights

As shown in Table 3, the mean litter size and litter weights of the rats infected post partum were significantly lower (P<0.05) than those of the uninfected control group on day 21 post delivery, even though there was no significant difference (P>0.05) in these parameters between the groups at birth and on days 7 and 14 post delivery.

3.4. Survival time

There was no significant difference (P>0.05) in the survival time between the rats infected post partum and their infected but unbred counterparts (Figure 1).

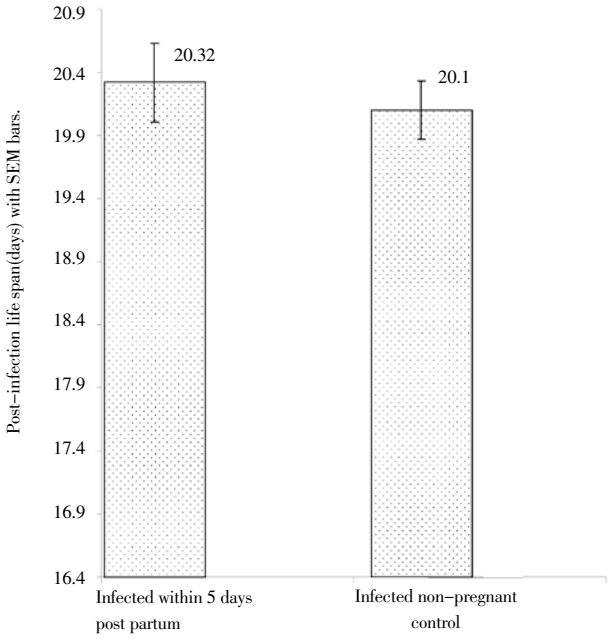

Figure 1. The survival time (in days) of rats infected with *T. brucei* 1–5 days post partum, compared with survival time of infected but unbred control group.

Table 3
The litter size and litter weight (g) of rats infected with *T. brucei* 1–5 days post partum compared with the litters of uninfected control group.

Time period	Mean litter size		Mean litter weight (g)	
	Infected	Uninfected	Infected	Uninfected
At birth	7.88±0.34	7.84±0.32	43.66±1.64	44.12±1.62
Day 7 post delivery	7.50±0.33	6.92±0.40	72.52±2.89	76.52±4.21
Day 14 post delivery	6.54±0.41	6.68±0.41	102.38±7.28	129.10±7.13
Day 21 post delivery	4.55[a]±0.61	6.64[b]±0.40	86.44[a]±11.46	186.90[b]±10.28

Results are presented as the means, with the standard error of the means indicated in parentheses. [ab]Different superscripts in a row denote significant difference between the means: P<0.05.

3.5. Trans-mammary infection

Screening of the blood of the pups from infected mothers showed no evidence of trans-mammary transfer of infection up till the weaning age of three weeks.

4. Discussion

The results of the present study showed significantly lower (P< 0.05) PCV values before infection, on day 4 post infection for the rats in groups 1 & 2 and the uninfected control group compared with the infected but unbred rats of group 3 respectively. This difference may be attributed to the relatively lower PCV values usually observed during late pregnancy and at the peri-parturient period[19] or to blood loss during parturition. It is known that the haemo-endothelial type of placentation that occurs in rats and other rodents is normally associated with bleeding following delivery due to high degree of invasion of the maternal endometrium by the foetal chorion during placentation[20]. The significantly lower PCV values of the two infected groups from day 8 post infection suggested that anaemia, which is a critical lesion of trypanosomosis[21-23] was beginning to be established.

It is evident from the study that the post parturient stress of suckling did not significantly alter the level of parasitaemia of animals that were infected post partum when compared

with that of the infected but unbred control group. This similarity in the level of parasitaemia may also account for the lack of significant difference in the PCV values of these two groups throughout the study period. This may also be responsible for the similarity in their survival time post infection.

Though no significant difference ($P>0.05$) was observed in the mean litter size and litter weight between the infected and uninfected groups at birth and on days 7 and 14 post delivery, there was a significant ($P<0.05$) difference in these parameters between the infected and uninfected group by the 21st day. This difference may be attributed to malnutrition arising from the overwhelming nature of trypanosomosis on the dam of this period, which may have significantly impaired the neuro-endocrine milk letdown mechanism[24-26], and by extension, adversely affected the nursing ability of the rats.

It is worthy of note that despite the fact that *T. brucei* belongs to the "humoral" group of trypanosomes[7], no evidence of transmammary transfer of infection was observed in this study.

In conclusion, *T. brucei* infection during the immediate post partum period could have serious negative health implications on the dam which could be detrimental to the growth and development of the offspring even in the absence of trans-mammary transmission of infection.

Conflict of interest statement

The authors declare no competing interest with the present work.

References

[1]Anene BM, Onah DN, Nawa Y. Drug resistance in pathogenic African trypanosomes; what hopes for the future? *Vet Parasitol* 2001; **96**: 83-100.

[2]Hide G, Tilley A. Use of mobile genetic elements as tools for molecular epidemiology (*T. brucei* rhodesiense). *Inter J Parasitol* 2001; **31**: 599-602.

[3]Hill EW, O'Gorman GM, Agaba M, Gibson JP, Hanotte O, Kemp SJ, et al. Understanding bovine trypanosomiasis and trypanotolerance: the promise of functional genomies. *Vet Immunol Immunopathol* 2005; **105**: 247-58.

[4]Kershaw WE. The African Trypanosomiases VII-IX. London:Allen and Unwin; 1970.

[5]Horst SHS. Trypanosomosis. In: Horst SHS. *Tropical animal health*. Dordrecht: Kluwer Academic Publishers; 1998. p, 152-69.

[6]Taylor K, Authie EML. Pathogenesis of animal trypanosomiasis. In: Maudlin I, Holmes PH, Miles MA. (ed). *The trypanosomiases*. Wallingford: CAB International; 2004.p,331-53.

[7]Soulsby EJL. Helminths, arthropods and protozoa of domesticated animals. 7th ed. London: The English Language Book Society and Bailliere Tindall; 1982.p,507 - 55.

[8]Llewelyn CA, Luckins AG, Munro CD, Perrie J. The effect of Trypanosoma congolense infection on the oestrous cycle of the goat. *Brit Vet J* 1987; **143**: 423-31.

[9]Ikede BO, Elhassan E, Akpavie SO. Reproductive disorders in African trypanosomiasis: a review. *Acta Trop* 1988; **45**: 5-10.

[10]Edeghere H, Elhassan E, Abenga J. Effects of infection with Trypanosoma brucei on different trimesters of pregnancy in ewes. *Vet Parasitol* 1992; **43**: 203-9.

[11]Sekoni VO. Reproductive disorders caused by animal trypanosomiasis: a review. *Theriogenology* 1994; **42**: 557-70.

[12]Ikede BO, Losos GJ. Pathological changes in cattle infected with Trypanosoma brucei. *Vet Pathol* 1972; **9**: 272-7.

[13]Ijagbone IF, Agbede SA. A case of congenital transmission of Trypanosoma brucei in mice. *Trop Vet* 2000; **18**: 37-8.

[14]Murray M, Murray PK, McIntyre WIM. An improved parasitological technique for the diagnosis of African trypanosomiasis. *Trans Roy Soc Trop Med Hyg* 1977; **71**: 326.

[15]Bennett JP, Vickery BH. Rats and mice - copulation and mating behaviour. In: Hafez ESE, editor. Reproduction and Breeding Techniques for Laboratory Animals. Philadelphia: Lea & Febiger; 1970.p,302-3.

[16]Ochiogu IS, Uchendu CN, Ihedioha JI. A new and simple method of confirmatory detection of mating in albino rats (Rattus norvegicus). *Anim Res Inter* 2006; **3**: 527-30.

[17]Coles EH. Veterinary clinical pathology. W.B. Philadelphia: Saunders & Company; 1986.

[18]Herbert WJ, Lumsden WHR. Trypanosoma brucei: A rapid matching method for estimating the host's parasitaemia. *Exp Parasitol* 1976; **40**: 427-31.

[19]Swenson MJ. Physiological properties and cellular and chemical constituents of blood. In: Swenson MJ.(ed.) *Duke's physiology* of *domestic animals*. 10th ed. London: Cornell University Press; 1984. p,15-40.

[20]McDonald LE. Pregnancy and parturition. In: McDonald LE, Pineda MH.(ed.) *Veterinary endocrinology and reproduction*. Philadelphia: Lea & Febiger;1989.p, 503-25.

[21]Anosa VO, Isoun TT. Serum proteins, blood and plasma volumes in experimental *Trypanosoma vivax* infection of sheep and goats. *Trop Anim Hlth Prod* 1976; **8**:14.

[22]Dargie JD, Murray PK, Murray M, Grimshaw WRT, McIntyre WIM. Bovine trypanosomiasis: the red cell kinetics of N'dama and Zebu cattle experimentally infected with *Trypanosoma congolense*. *Parasitol* 1979a; **78**: 271.

[23]Dargie JD, Murray PK, Murray M, McIntyre WIM. The blood volumes and erythrokinetics of N'dama and Zebu cattle experimentally infected with Trypanosoma brucei. *Res Vet Sci* 1979b; **26**: 245.

[24]Lincoln DW, Paisley AC. Neuroendocrine control of milk ejection. *J Reprod Fertil* 1982; **65**: 571-86.

[25]Hatton GI. Emerging concepts of structure-function dynamics in adult brain: The hypothalamo-neurohypophysial system. *Prog Neurobiol* 1990; **34**: 437-504.

[26]Crowley WR, Armstrong WE. Neurochemical regulation of oxytocin secretion in lactation. *Endocr Rev* 1992; **13**: 33-65.