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ARTICLE

Effects of *Trypanosoma brucei* and *Heligmosomoides bakeri* Infections on Water Consumption of Lactating Albino Mice and the Viability of their Pre-Weaned Offspring

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SUMMARY

The effects of single and/or concurrent H. bakeri and T. brucei infections on water consumption of lactating and non-lactating mice were investigated. Pregnant mice were grouped into four (A, B, C and D) comprising of six animals per group. Groups A, B and C were either infected with H. bakeri or T. brucei alone or with both parasites together. Group D served as pregnant uninfected control while a different group (E) was used as non-pregnant and uninfected control. Packed cell volumes (PCV), faecal egg counts (FEC), worm burden and water-consumption of the mice were determined. Mean live-weights of surviving offspring and their numbers were recorded. Infected animals had lower PCV compared to the controls. However, lactating mice infected with both parasites had the least PCV. Mortalities occurred in the dual-infected groups. Worm burden and FEC of mice with concurrent infections were significantly higher than that of the H. bakeri-only infected mice. Lactating mice consumed significantly more water than non-lactating mice. Uninfected and H. bakeri-only infected mice had heavier, healthy-looking and greater numbers of pups than their conjointly-infected counterparts that had fewer wrinkled-bodied pups with more early-mortalities following parturition. Drawing from these results, it is therefore necessary to promptly control diseases in pregnant and lactating animals and provide water adlibitum to lactating animals.

Keywords: Water consumption, Lactating mice, Trypanosoma brucei, Heligmosomoides bakeri, concurrent infection

INTRODUCTION

Field and experimental studies have revealed the negative effects of trypanosome infections on livestock (Faye et al., 2005). Experimental Trypanosoma congolense infection has been shown to lower the productivity of WAD goats causing significant numbers of abortions and still births and as well as mortalities and reduced growth (Goossens et al., 1997). Also of great importance is the potential of trypanosomes to down regulate the immune responses of their hosts to many pathogens including

gastrointestinal nematodes of domestic animals (Onah and Wakelin 1999; Chiejina et al., 2005). Likewise, gastrointestinal nematode infections contribute immensely to the poor health and productivity of animals in the tropics (Idika et al., 2012). It has been demonstrated that Lactating animals are more susceptible to infections with helminth parasites compared to the non-lactating ones and the increased susceptibility of lactating animals had been attributed to immune-suppression (Chartier et al., 1998; Keyyu et al., 2006).

Maintenance requirements of pregnant and lactating animals are known to be higher than their non pregnant/lactating counterpart (Mandok et al., 2008). The increase in maintenance requirements of pregnant and lactating animals is believed to be partially due to increases in relative weights and metabolic activities of vital organs during such reproductive activities (Jacobs et al., 2011). Water intake of North American lactating female porcupines (Erethizon dorsatum) have been shown to be 16% greater than that of their non-lactating ones (Holmes 1993). Systemic relaxin has been shown to promote moderate water consumption during late pregnancy in rats (Omi et al., 1997).

This study therefore, was designed to compare the water consumption of lactating and non-lactating mice with single and/or concurrent infections with H. bakeri and T. brucei brucei and to compare the survivability and performance of the offspring of infected and non-infected females.

MATERIALS AND METHODS

Experimental animals and design

Thirty (30) adult female mice were used for the study. Twenty four of the mice were introduced to males for mating. Gross observation of grey to yellowish protein coagulates (remnants of the copulatory plug) on vaginal smears of mated females made on clean glass slides as described by (Ochiogu et al., 2006) were made to confirm mating. Pregnant mice were then grouped into four groups (A, B, C and D) comprising six animals per group. Groups A, B and C were either infected with H. bakeri or T. brucei alone or with both parasites together as shown in Table 1. Group D served as pregnant uninfected control while a different group (E) was used as non-pregnant and uninfected control. Infection with T. brucei was done five days before H. bakeri infection in the case of concurrent infection.

Infection of mice with H. bakeri

Infective larvae (L3) of H. bakeri harvested from faecal cultures prepared with faeces obtained from donor mice carrying H. bakeri infection were used for the infections. To set up faecal cultures, harvested faeces from the H. bakeri infected mice were first crushed, suspended in water and sieved with a coffee strainer into a 60 ml tubes. The tubes containing the filtrates were centrifuged and the sediment smeared on filter papers placed in petri dishes. The petri dishes containing the faecal smears were kept in a refrigerator for seven (7) days after which water was poured into the dishes just enough to cover the filter papers and the culture kept for one hour to allow the larvae move into the water. The water now containing the larvae was carefully poured into test tubes and stored at 4 °C in a refrigerator and used within 2 weeks of recovery from cultures. Prior to administration of the larvae to the experimental animals, counts were performed to determine the infecting doses. All the mice meant for H. bakeri infection received 100 L3 as described by Fakae (1993).

Infection of the experimental mice with T. brucei brucei

Trypanosoma brucei brucei (an isolate from N'dama cattle from Federer village in Plateau State, Nigeria) was used in the study. It was obtained from the Nigerian

Institute of Trypanosomiasis and Onchocerciasis Research (NITOR) Vom, Plateau state, Nigeria. The parasites were inoculated and subsequently maintained by serial passage in laboratory mice. Parasites harvested from the infected donor mice were used to inoculate the experimental mice intraperitoneally with 6.0 x 10^6 trypanosomes per mouse as shown in Table 1. The parasites were estimated using the rapid matching method of Herbert and Lumsden (1976).

TABLE I: Experimental design and infection protocol.

Groups	Group description	Day of Tb infection	Days of Hb infection	Dose of Tb	Dose of Hb
A	Pregnant mice infected With Tb	-5	0	6 x 10^6	0
B	Pregnant mice infected With Tb & Hb	-5	0	6 x1 0^6	100L_3
C	Pregnant Mice infected With Hb	_	0	0	100L_3
D	Uninfected Pregnant mice	_	0	0	0
E	Non-pregnant Uninfected mice	_	0	0	0

Hb=*H. bakeri,* Tb=*Trypanosoma brucei,* L_3 = infective larvae

Determination of Packed cell volume (PCV)

Packed cell volume (PCV) was determined by the haematocrit method (Dacie and Lewis 1975) beginning from day zero (D0) of the experiment and every four days thereafter.

Faecal egg counts (FEC)

Faecal egg counts, expressed as eggs per gram (epg) of faeces, was carried out thrice weekly following patency on freshly collected faecal samples from individual mice using centrifugal floatation in saturated salt (NaCl) solution and where appropriate by the modified McMaster technique as described by MAFF (1977).

Necropsy and worm counts

Post mortem examination was carried out on all the experimental animals on day 24 as described by Fakae (1993). Mice were humanely sacrificed using diethyl ether and their small intestine excised longitudinally, submerged in Hank's balanced salts solution with a fine nylon thread and incubated at 37°C in universal bottles. Within 2-3 hours the worms had migrated out of the lumen and dropped at the bottom of the bottles for collection, preservation and counting.

Water consumption

Water consumed by the various groups was determined daily and the weekly average of each group calculated. The amount of water consumed was determined by measuring the difference in the volume of water given and that left after 24 hours using a measuring cylinder.

Litter weight

The average litter weight (g) at birth of the pubs born to each female was determined soon after birth by weighing their respective litters and their average per group calculated.

Statistical analysis

Statistical analysis was conducted using SPSS version 15 for Windows. Parameters recorded on more than a single day (FEC,

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Statistical analysis was conducted using SPSS version 15 for Windows. Parameters recorded on more than a single day (FEC, parasitaemia and PCV) were analyzed by repeated measure ANOVA in General Linear Model (GLIM). Where data conformed to normal distributions, analysis was by ANOVA in GLIM on raw values and the results were summarized as arithmetic means with standard errors of the mean (SEM). Where data did not conform to normal distribution, an appropriate logarithmic transformation was adopted prior to analysis and all residuals for ANOVA checked for appropriate normal distribution. The data were

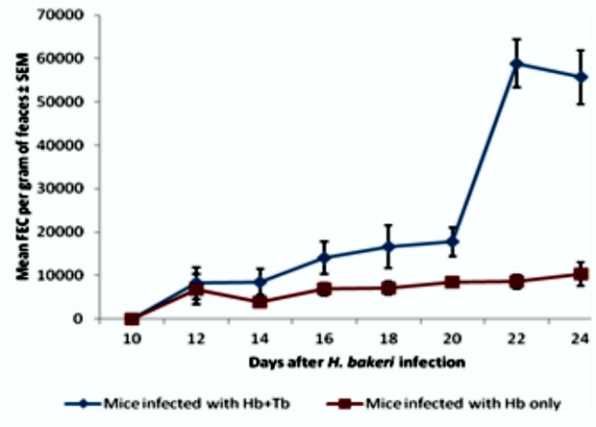

Figure 1: Mean Faecal egg counts of mice infected with either H. bakeri alone or with both H. bakeri and T. bruceiFigure 1: Mean Faecal egg counts of mice infected with either H. bakeri alone or with both H. bakeri and T. brucei

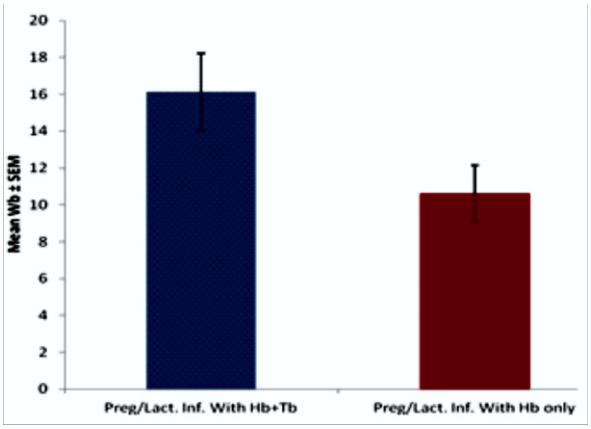

Fig 2: Mean worm burden of pregnant/lactating mice infected either with H. bakeri alone or with both H. bakeri and T. brucei.

shown as mean log values ± SEM. Probabilities (P) of 0.05 or less were considered significant.

RESULTS

Parasitological responses Trypanosoma brucei infection: The prepatent period for Tb in mice infected with Tb only (Grp A) and Tb + Hb (Grp B) was 5-6 days, with peak parasitaemia occurring at 10 – 12 days. Analysis by repeated measures ANOVA indicates that the difference between the mean parasitaemia of the two groups was not significant (P > 0.05).

Heligmosomoides bakeri infection: The mean pre-patent period as shown by the occurrence of H. bakeri eggs in faeces was 10 days for the T. brucei bruei and H. bakeri concurrently infected mice and 11.0 ± 1.0 days (range: 10 – 12 days) for mice infected with H. bakeri only. The FEC of both groups (B and C) rose sharply from D10 to D12 following patency and no significant difference (P > 0.05) was observed between their mean Log 10(FEC + 1) up to D14 post infection. Thereafter, the mean FEC of mice concurrently infected with H. bakeri and T. brucei continued to rise and was significantly higher (P ≤ 0.05) than those of mice infected with H. bakeri only (Fig 1). The worm burden of the mice had the same trend as the FEC. The mean WB of the mice concurrently infected with H. bakeri and T. brucei was significantly higher (P = 0.05) than those of H. bakeri-only infected mice (Fig 2).

Packed cell volume (PCV)

Figure 3 shows the changes in the PCV of the mice. Among the uninfected groups, the PCV of the non-pregnant uninfected mice were fairly constant throughout the duration of the experiment whereas that of the pregnant uninfected mice showed a decline from D0 to

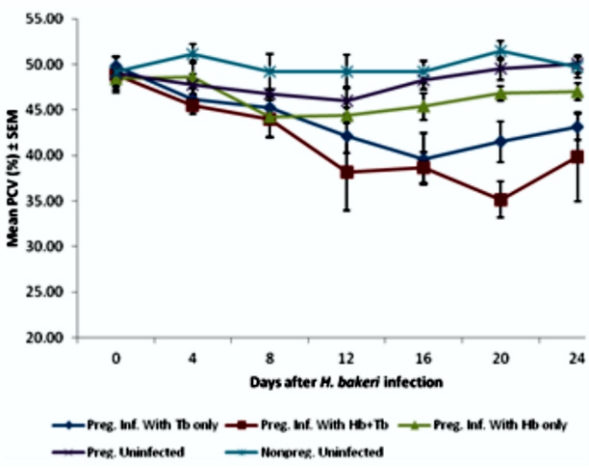

Fig 3: Mean packed cell volume (%) of uninfected-pregnant/lactating mice and pregnant mice infected either with H. bakeri alone, T. brucei alone or both H. bakeri and T. brucei.

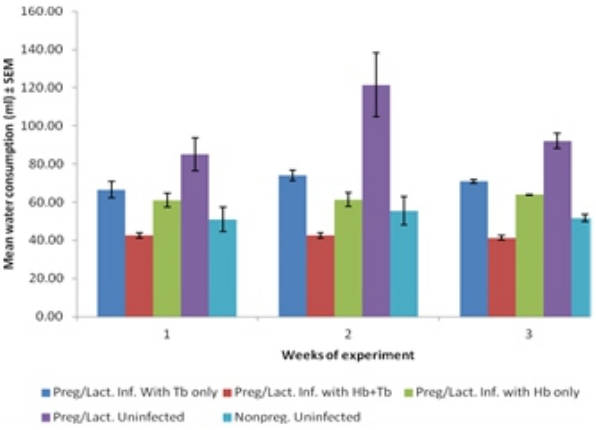

Fig 4: Mean weekly water consumption (ml) of uninfceted-lactating mice and lactating mice infected either with H. bakeri alone, T. brucei alone or both H. bakeri and T. brucei.

D12 before rising to become similar with that of the non-pregnant uninfected mice. The mean PCV of the non-pregnant uninfected mice were significantly higher (P > 0.05) than those of the infected mice throughout the duration of the experiment. Among the infected mice, the mean PCV of mice infected with only H. bakeri were comparable with that of the pregnant uninfected mice up to D12 after infection with H. bakeri, thereafter, it became significantly lower (P > 0.05). There was a steady decline in the mean PCV of mice either infected with T. brucei only or

Performance and Survivability of offspring and their dams

Uninfected- and H. bakeri-only infected mice had more pups that were heavier, smooth-bodied and healthier-looking than those of the T. brucei-only and T. brucei and H. bakeri-conjointly infected mice that had fewer wrinkle-bodied pups with some still birth among them and early-mortalities following parturition. The mean average litter weight at birth of the pubs born to dams that were either infected with T. brucei only or concurrently with H. bakeri was significantly lower ($P < 0.05$) than those of H. bakeri-only infected and the pregnant uninfected mice (Fig 5).

DISCUSSSION

The results showed that the mice conjointly infected with H. bakeri and T. brucei suffered the infection more than the T. brucei- or H. bakeri-only infected mice. It believed that the trypanosome may have compromised the ability of the mice to control the nematode infection as was evidenced by the higher worm burden and faecal egg counts/gram of faeces (FEC) observed among the mice concurrently infected with both parasites. Concurrent infections with other related or unrelated parasites or pathogens have been shown to markedly compromise the ability of an animal to control a given parasitic infection (Kaufmann et al., 1992; Goossens et al., 1997; Chiejina 2001). However, a comparable level of parasitaemia and PCV was observed between the T. brucei-only and T. brucei and H. bakeri concurrently infected mice, suggesting that H. bakeri did not significantly alter the responses of the mice to T. brucei infection.

Pregnancy and lactation among other factors have being shown (Omi et al., 1997; Lardy and Stoltenow 1999) to influence water consumption in livestock. It is reported that the maintenance requirements of pregnant and lactating animals are higher than their non pregnant and lactating counterpart (Mandok 2012). This was also true in our findings as the water intake observed among the lactating uninfected mice was significantly higher than their non-lactating counterparts (non pregnant uninfected). It has been demonstrated (Canas et al., 1982) using rats that, the increases in maintenance requirements of pregnant and lactating animals are partially due to increases in relative weights and metabolic activities of vital organs during such reproductive activities. Dairy cattle are known to consume significantly more water compared to beef cattle because the former requires a lot of water for their milk production (Lardy and Stoltenow 1999; The Merck Veterinary Manual 1986). Dairy cattle have been shown to consume averagely 20 gallons of water compared to 10 gallons per head per day for beef cattle (The Merck Veterinary Manual 1986). Large drop in egg production are known to occur due to water deprivation (Jacobs 2011). Water intake of the North American lactating female porcupine (Erethizon dorsatum) has been shown to be 16% greater than that of their non-lactating female counterparts (Farrel and Christian 1987).

However, it was observed in this study that both T. brucei and H. bakeri infections significantly decreased the water consumption of the mice. This depressive effect on water consumption was observed most in the group infected concurrently with T. brucei and H. bakeri. The result also showed that when singly infected, H. bakeri had more depressive effect on water consumption than T. brucei. This effect on water consumption is believed to be related to decrease feed intake of the mice as a result of the infection as both gastro-intestinal nematodes (Holmes 1993) and trypanosomes (Wassink et al., 1993) infections have been shown to suppress feed intake in animals. Studies (Bachmanov et al., 2001; Selman et al.,

2001) have shown that feed and water intake are interrelated.

In this study, the pregnant non-infected and H. bakeri-only infected mice had viable offspring that survived and performed better that those of the trypanosome-only and trypanosome plus nematode infected mice. Also, the uninfected mice were seen gathering, grooming, and suckling their pups more often than the mice with trypanosome infections. These findings underscore the earlier established importance of trypanosomosis in reproduction (Ochiogu et al., 2008; Pathak 2009).

In conclusion, these results reiterate the importance of controlling parasitic infections in pregnant and lactating animals and the need to provide improved nutrition which consequently improve water intake

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