Full Length Research Paper

Influence of pregnancy and lactation on the parasitological and clinico-pathological responses of out-bred albino mice to *Heligmosomoides bakeri* infection administered at different trimesters of pregnancy

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Influence of pregnancy and lactation on the parasitological and some clinico-pathological responses of out-bred albino mice to single doses of *Heligmosomoides bakeri* (H.b) were studied. Fifty six pregnant and ten non-pregnant mice were used for the study. The pregnant mice were either infected in the first, second or third trimester depending on the experimental group and were either allowed to suckle or not following parturition. The ten non-pregnant mice served as uninfected controls. Faecal egg counts (FEC), bodyweights (BW), packed cell volume (PCV) and worm burdens (WB) were recorded. Both lactating and non-lactating mice infected in the first trimester had significantly higher WBs ($F_{2,49}$ = 5.242, P = 0.001) and FECs ($F_{2,37}$ = 4.314, P = 0.001) in comparison to their counterparts infected in the second and third trimesters. Generally, the WB of lactating mice was only marginally higher than those of non-lactating mice in the first and third trimesters. The FECs of non-lactating mice were generally higher than those of lactating mice. The PCV of lactating mice was lower than those of both non-lactating and uninfected control mice. The body weights of both lactating and non-lactating mice (infected) were lower compared to those of the uninfected control mice. No periparturient rise in FEC was observed nor did lactation influence FEC.

Key words: Pregnancy, lactation, *Heligmosomoides bakeri*, outbred albino mice, parasitological and clinico-pathological responses.

INTRODUCTION

The epidemiology of gastrointestinal (GI) nematode infections is not fully understood although the "wormy world" awaits to be dewormed and worms controlled in an acceptable manner commensurate to profitable livestock production. The complex nature of the epidemiology of nematode infections is obvious and has been shown to be affected by factors such as the infective dose of the parasite, age of the host and sex of the host. Pregnancy is said to be one of the factors that could alter the response pattern of some animal species to GI nematode infections (Soulsby, 1982). The physiology of both pregnant animals and pregnant women has been shown to place them on the disadvantage with regard to response to both helminth and other infections (Soulsby, 1982; Urquhart, 1988; Rohan, 1990; Denise et al., 2006). This observation has been exhibited by the periparturient rise (PPR) in faecal egg counts (FEC) in GI nematode infections (Soulsby, 1982).

Pregnancy and lactation have been reported to depress the immune response to many helminths such as *Nippostrongylus brasiliensis* infection in rats (Connan, 1970, 1972) resulting in increased establishment,

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Table 1. Experimental protocol: Infection of pregnant, lactating and non-lactating mice with 100 L3 *H. bakeri* at different trimesters of pregnancy.

Group	Number of mice	Trimester of pregnancy (Day)	Age of mice (Weeks)	Dose of Hb L3	Day of necropsy (Post infection)
Infected/nonlactating.	8	First (5)	10	100	60
Infected/lactating	8	First (5)	10	100	60
Infected/nonlactating	10	Second (12)	10	100	60
Infected/lactating	10	Second (12)	10	100	60
Infected/nonlactating	10	Third (17)	10	100	60
Infected/lactating	10	Third (17)	10	100	60
Uninfected	10	Nonpregnant	10	0	60

Hb = *Heligmosomoides bakeri*, L3 = infective larvae, nonlactat. = non-lactating, Infected/nonlactating = Infected mice that were not lactating, Infected/lactating = Infected mice that were lactating, Uninfected = uninfected control mice.

fecundity, survival of adult worms and activation and development arrested larvae. The reduced immune competence due to lactation is said to be best demonstrated in rats infected during the first two weeks following parturition and was dependent on suckling because rats that had their pups removed after birth did not harbour extended infections. The immune suppression was serious enough to interfere with the expression of immunity in rats sensitized by previous infections (Connan, 1972). The increment in prolactin concentration in the course of lactation affects the differentiation of lymphoid cells (Kelly and Dineen, 1973).

The suckling reflex is said to be a requisite for the sustenance of high levels of blood prolactin, which is the hormone incriminated for the immuno-suppressive effect of pregnant individuals and the subsequent increase in FEC of nematode eggs in the faeces of the hosts in the periparturient period (Kickvn, 2010; Turner and Bagdara, 1971). Apart from the PPR in helminth infections, pregnancy has also been shown to enhance the prevalence and pathology of other infections such as malaria in pregnant women (Urquhart et al., 1996; Soulsby, 1982; Larocque et al., 2006, Denise et al., 2006). Furthermore,

among maternal infections, cytomegalovirus, rubella, toxoplasma, and *Herpesvirus hormonis* are common and the clinical picture is reported to depend on the severity, time and duration of the infection, being especially vulnerable in the first trimester of pregnancy (Rohan, 1990). Anaemia, malaria and intestinal helminths often co-exist and are potentially serious conditions of pregnant women in the developing economies/countries and the World Health Organisation (WHO) recommends anthelmintic drugs for the control of intestinal nematodes such as hookworms during pregnancy (WHO, 1996, 1999).

Prolactin whose concentration increases during pregnancy and post-parturitioin, is an immmuno-suppressor and pregnant and lactating mice have been shown to have decreased immunocompetence compared with that of non-reproducing mice (Ferguson et al., 1982; Sulila and Mattson, 1990; Medina et al., 1993). It is known to suppress rejection of *N. brasiliensis* in Lewis strain of rats (Kelly and Dinnen, 1973). Due to rise in concentration of prolactin during pregnancy (Hwang et al., 1971), most pathogens gaining access to such hosts at such periods may establish greatly, inflicting greater pathology to their hosts. It has been reported that the

prevalence of infection and parasite density are highest during the first half of pregnancy (Okoko et al., 2003). It was therefore necessary to examine in the present experiment how the trimester of pregnancy and lactation would affect the parasitological and some clinico -pathological responses of mice to *H. bakeri* single primary infections.

MATERIALS AND METHODS

Animals and their management

Ten-week old female mice with average weight of 29 g, bred in the Department of Veterinary Parasitology and Entomology, University of Nigeria, Nsukka were used for the experiment. The experimental protocol is shown in Table 1.

Mating

Male and female mice were kept in the ratio of 1:5 on D0 of the experiment and were assumed all to be mated within five days. Mating was confirmed according to the method of Ochiogu et al. (2006). This comprised of gross observation of grey to yellowish protein coagulates (remnants of the copulatory plug) on vaginal smears of mated females made on clean glass slides. Pregnancy was further confirmed by rapid weight gains and distended bellies.

Infection of mice with *H. bakeri*

The mice were infected orally with 100 *H. bakeri* infective larvae (L3) suspended in 200 µl of distilled water. To obtain accurate dosage, properly mixed known volumes of larval suspension were administered to properly restrained mice using automatic micropipette (Finnpippette®; Labsystems Oy, Helsinki, Finland) adapted to take a blunt and slightly curved 19-gauge needle (Fakae, 2001).

Parturition and lactation

Not all infected animals were allowed to suckle the litter, rather for mice of each trimester, 50% were allowed to suckle following parturition while the litter of the other 50% were fostered to those lactating mice (foster dams).

Haematology

Packed cell volume (PCV) was carried out weekly from day zero (D0) till the end of the experiment. Mice were bled from the tail directly into heparinized capillary tubes (Camlab Ltd, Cambridge).

Body weight

Mice were weighed weekly from the first day of each experiment using a desktop balance (Sartorius GMBH Gottingen Germany).

Faecal collection

Faeces for faecal analysis were collected from individual mouse by placing it in large faecal collection cups. 1 to 2 min was enough for each well-fed mouse to pass out enough faecal pellets for faecal analysis or culture as the case may be. Mouse picked from their cages without much excitement would not lose the rectal pellets to their cages yet, but would immediately pass them out in the cups following the grasping excitement.

Faecal egg counts

One gram of faeces collected from each mouse was dispersed in 15 ml of saturated sodium chloride solution of specific gravity 1.200. The suspension was passed through a coffee strainer and made up to 45 ml with additional salt solution. Well-mixed aliquots were counted in a standard McMaster counter slide (Hawksley, England) and expressed as eggs per gram (epg) of faeces (MAFF, 1997). Egg count was multiplied by 150 to get the FEC.

Necropsy/worm burden

Post mortem worm counts procedures were according to the methods of Fakae (1993) and Ngongeh (2008, 2011). Briefly, each mouse was sacrificed with diethyl-ether and the gastrointestinal tract was quickly removed. Up to three quarters or the entire length of the small intestine starting from the duodenum was opened by cutting along its longitudinal axis with a pair of fine scissors. The adult worms from the intestine were recovered by suspending each intestine on a fine thread and dipping into Hanks balanced salt

solution (HBSS) in a universal bottle, and the thread supported by fastening its free ends between the universal bottle and its cap, and then incubated at 37°C. This arrangement allowed the worms to drop freely to the bottom of the bottle after leaving the intestine. Within 2 to 3 h all worms would have migrated into the saline. The intestine was then discarded, after little agitation to ensure that no worm was being trapped by any two apposed sections of the gut, and fresh saline added. After removal of the intestine the incubation of the worm suspension continued overnight (20 h) to ensure complete disentanglement of the worms. At the end of the overnight incubation saturated sodium chloride was added to the Hanks saline containing the worms to make up a 30% v/ v solution. The worms then died within 30 min of this treatment, relaxing the tight spiral coils characteristic of the live worms, thus making counting easier. The volume of the suspension was reduced to 5 ml and poured into a clean ruled Petri dish. All the worms present in each sample were counted individually noting their sexes.

Statistical analysis

This was carried out using SPSS version 12.0.1 for Windows, as described by Behnke et al. (2006). Where data conformed to normal distribution, analysis was by Analysis of Variance (ANOVA) in general linear model (GLIM) and results were summarised as arithmetic means with standard errors of means (SEM). Those parameters, which were recorded on more than one occasion namely, body weights, FEC, and PCV were analysed by repeated measures ANOVA (rmANOVA) in GLIM. Where data did not conform to normal distribution appropriate logarithmic transformations namely, $Log_{10}(X+25)$ for FEC (Chiejina et al., 2005) and $Log_{10}(X+10)$ for worm counts (Fakae et al., 2002; Chiejina et al., 2005) were adopted prior to analysis and all residuals for ANOVA were checked for approximately normal distribution. These data are summarised as mean log values ± SEM. Correlations between variables were analysed by Spearman's Rank order Test. Probabilities of 0.05 or less were considered significant.

# RESULTS

Parturition

Parturition started on D21 after mating. All pregnant mice whelped within four days from the initiation of parturition. Mice infected during the first trimester (D5 of the experiment) had patent infection 3 days before the initiation of parturition, while the second (D13 of the experiment) and third (D18 experiment) trimester-infected ones had patent infections 2 and 7 days after parturition, respectively. Fostered mouse pups were widely accepted by their foster dams. Litter sizes varied from 5 to 10 pups per dam and this was advantageous in that the lower litter sizes of foster mothers created suckling opportunities for the fostered pups. Over 90% of the pups survived.

Faecal egg counts

There was marked variation in FEC during each phase of pregnancy/lactation. The FECs of both lactating and non-lactating mice infected in the first trimester were
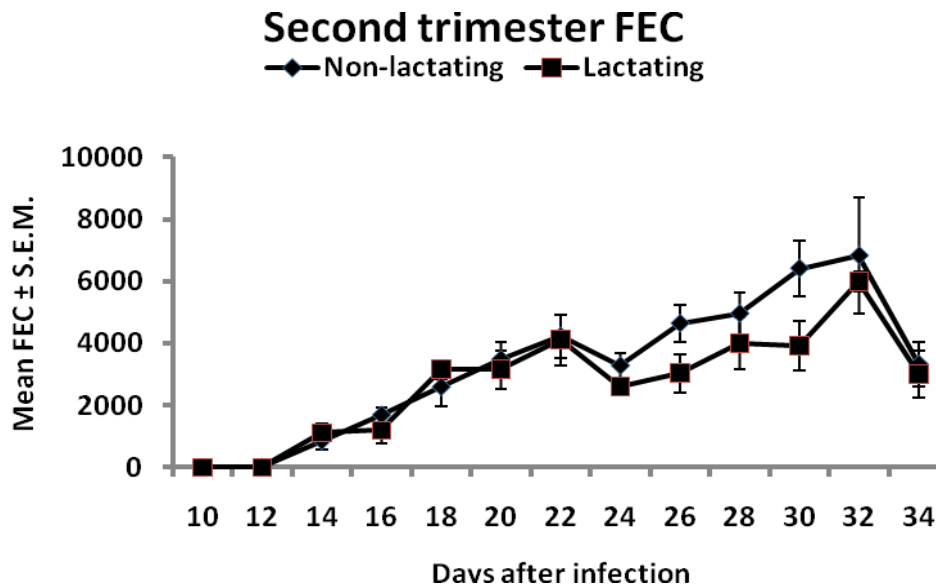
First trimester FEC
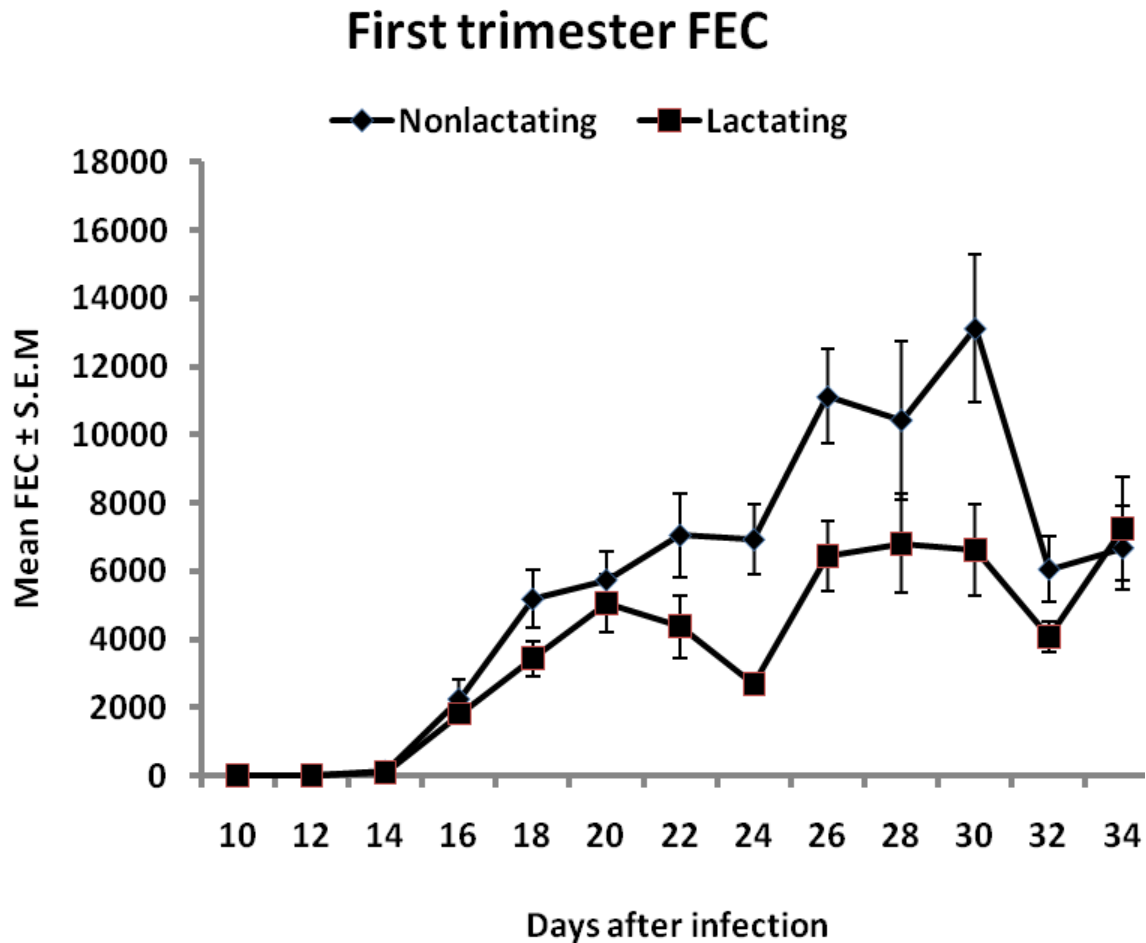
Figure 1a. Mean faecal egg counts (FEC) of lactating and non-lactating mice infected with 100 L3 *Heligmosomoides bakeri* in the first trimester of pregnancy.

significantly higher than those of their counterparts infected in the second and third trimesters ($F_{2,37}$ = 4.314, P = 0.001). Mice infected in the second trimester had the least FECs while mice infected in the third trimester had moderate FECs (Figure 1a, b and c). The main effect of lactation on FECs was not significant ($F_{1,37}$ = 0.319, P = 0.576). Although lactation had no significant effect on FEC, FECs were generally higher in non-lactating than in lactating mice during the first and second trimesters (Figure 1a and b). There was virtually no difference in faecal counts of these two groups during the third trimester. Overall more worm eggs were passed out by the first and third trimester-infected mice. FECs of the mice changed significantly with time ($F_{12,37}$ = 158.6695, P = 0.001).

Worm burden

The main effect of infecting mice at different trimesters on WB was highly significant ($F_{2,49}$ = 5.242, P = 0.001). WBs of lactating and non-lactating mice infected in the first trimester were significantly ($F_{2,49}$ = 5.242, P = 0.001)

higher than those of lactating and non-lactating mice infected in the second and third trimesters (Figure 1d). The WB of lactating mice infected in the first trimester was apparently higher than the WB of non lactating mice infected in the first trimester (P = 0.859), but significantly (P = 0.006) higher than the WB of lactating mice infected in the second trimester, significantly (P = 0.001) higher than the WB of non lactating mice infected in the second trimester and significantly (P = 0.001) higher than the WB of lactating mice infected in the third trimester. Though, the WB of lactating mice infected in the first trimester was higher than the WB of non lactating mice infected in the third trimester but the difference was not significant (P = 0.159). The WB of lactating mice infected in the second trimester was slightly higher than the WB of non lactating mice infected in the second trimester (P = 0.527). The WB of lactating mice infected in third trimester was significantly (P = 0.033) lower than the WB of non lactating mice infected in the third trimester. Mice infected during the second trimester generally had moderate WB compared to their counterparts infected in first and third trimesters (Figure 1d).

Second trimester FEC

Figure 1b. Mean faecal egg counts (FEC) of lactating and non-lactating mice infected with 100 L3 *Heligmosomoides bakeri* in the second trimester of pregnancy.

Packed cell volume

PCV varied significantly ($F_{5,16}$ = 4.241, P = 0.012) with time (Figure 1e,f and g). However, the trimester in which the mice were infected had no significant influence on the PCV ($F_{2,16}$ = 0.347, P = 0.564). The lactation status of mice also had no significant effect on their PCV regardless of the trimester in which they were infected ($F_{1,16}$ = 0.054, P = 0.819). The mean changes in PCV of mice infected during the first, second and third trimesters are presented in Figure 1e, f and g.

Body weights

Body weights of mice varied significantly with time ($F_{5,16}$ = 13.573, P = 0.001). Body weights of pregnant animals increased to a peak on D14 and dropped sharply on D21 before rising on D42, with lactating mice maintaining higher weights in comparison to their non lactating counterparts (Figure 1h, i and j). However, the main effect of lactation on body weights was not significant ($F_{1,16}$ = 0.266, P = 0.613). Body weights did not vary significantly with the trimester of pregnancy ($F_{2,16}$ = 0.364, P = 0.555). All mice gained weight by the end of the study on D42.

DISCUSSION

The results of this experiment are summarised as follows: (1) Both lactating and non-lactating mice infected in the first trimester had significantly higher WBs and FECs in comparison to their counterparts infected in the second and third trimesters. (2) Generally, the WB of lactating mice was only marginally higher than those of non-lactating mice in the first and third trimesters. (3) The FECs of non-lactating mice were generally higher than those of lactating mice. (4) The PCV of lactating mice was lower than those of both non-lactating and uninfected control mice. (5) The body weights of both lactating and non-lactating mice (infected) were lower compared to those of the uninfected control mice.

Pregnant and lactating mice have been shown to have decreased immune-competence compared to non-reproducing mice (Ferguson et al., 1982; Sulila and Mattson, 1990; Medina et al., 1993). Prolactin is a known immune suppressor (Kelly and Dineen, 1973). Its concentration increases from the first trimester to the third trimester and remains for 1 to 2 weeks post-partum (Hwang et al., 1971; Dusza and Krzymowska, 1981). For example, in sows plasma prolactin levels ranged from 4.4 to 13.0 ng/ml during pregnancy. It further increased to 20.3 and 103.4 ng/ml two days and one day respectively before farrowing (Dusza and Krzymowska, 1981; Kendall et al., 1982). The values ranged from 124.2 to 147.3 ng/ml during farrowing. On the 5th day of lactation the prolactin levels had fallen to 43.1 ng/ml. Although the level of this hormone was not measured in the study it is possible that during the first trimester an increase in prolactin may have been responsible for the higher FEC and WB of the first trimester infected mice. It has been reported that the prevalence of infection and parasite density are highest during the first half of pregnancy (Okoko et al., 2003).

It was thought that the likely high levels of prolactin in the periparturient mice would lead to a periparturient relaxation in immunity and that this could in turn give rise
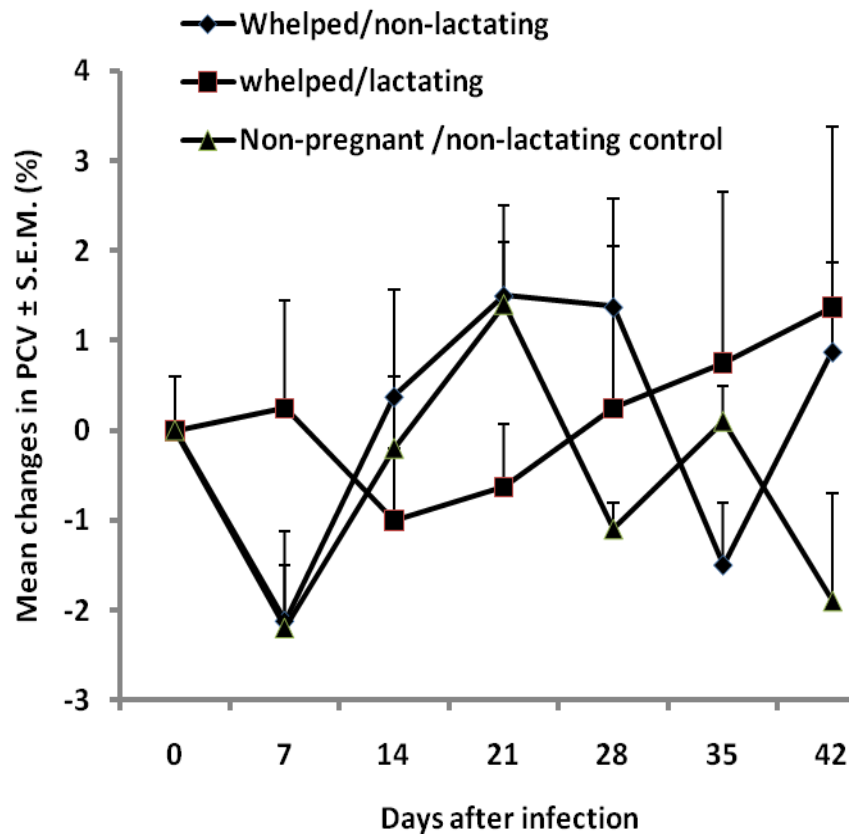
Third trimester FEC
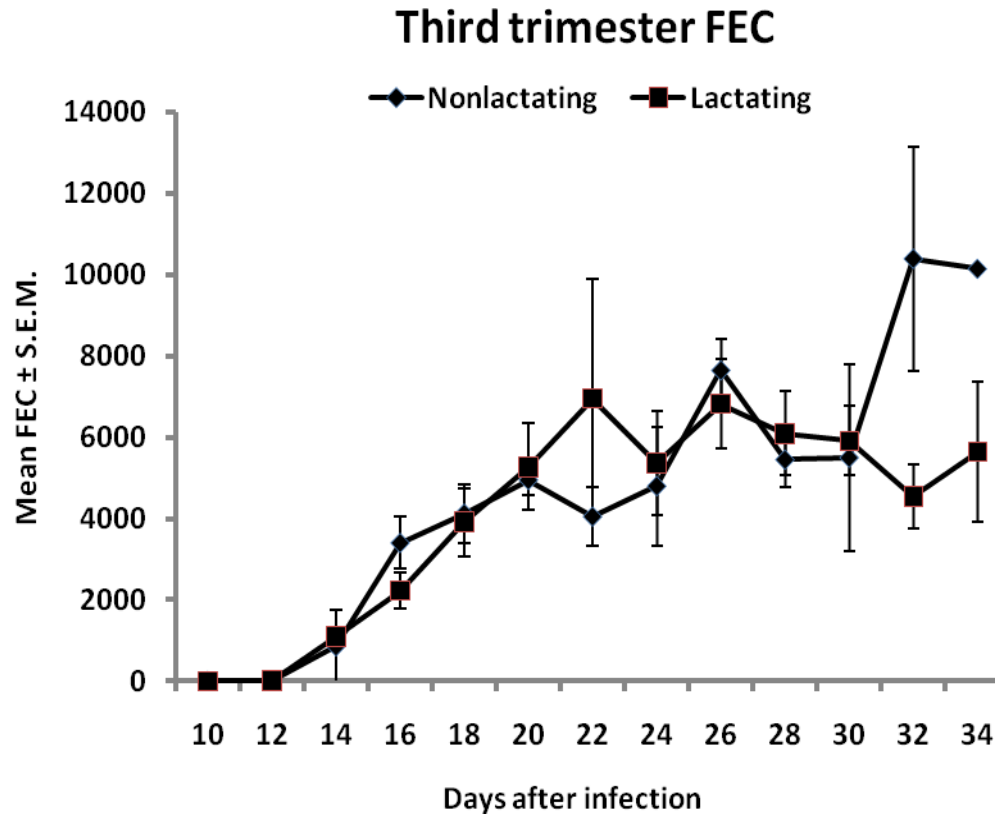
Figure 1c. Mean faecal egg counts (FEC) of lactating and non-lactating mice infected with 100 L3 *Heligmosomoides bakeri* in the third trimester of pregnancy.
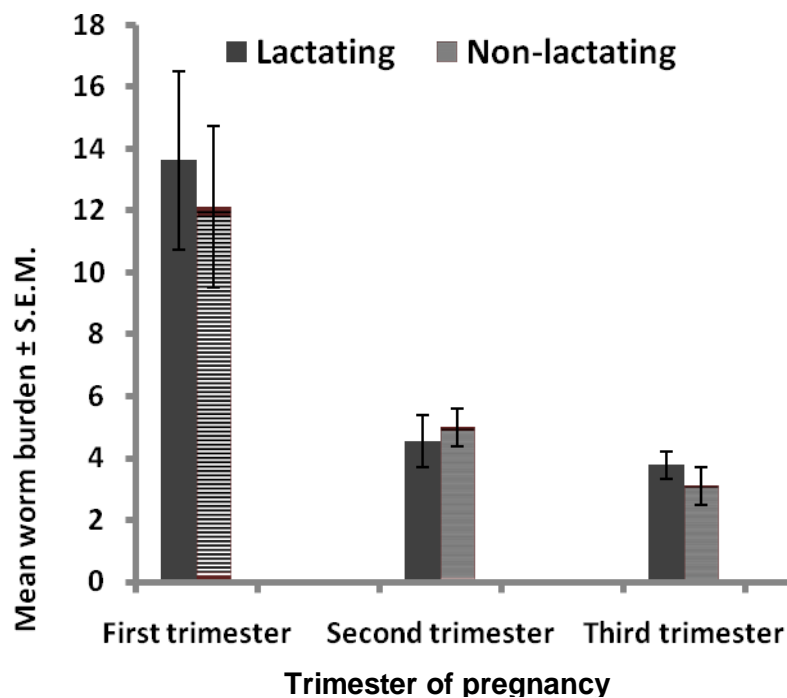
Figure 1d. Mean worm burdens of lactating and non-lactating mice infected with 100 L3 *H. bakeri* in the first , second and third trimesters of pregnancy.
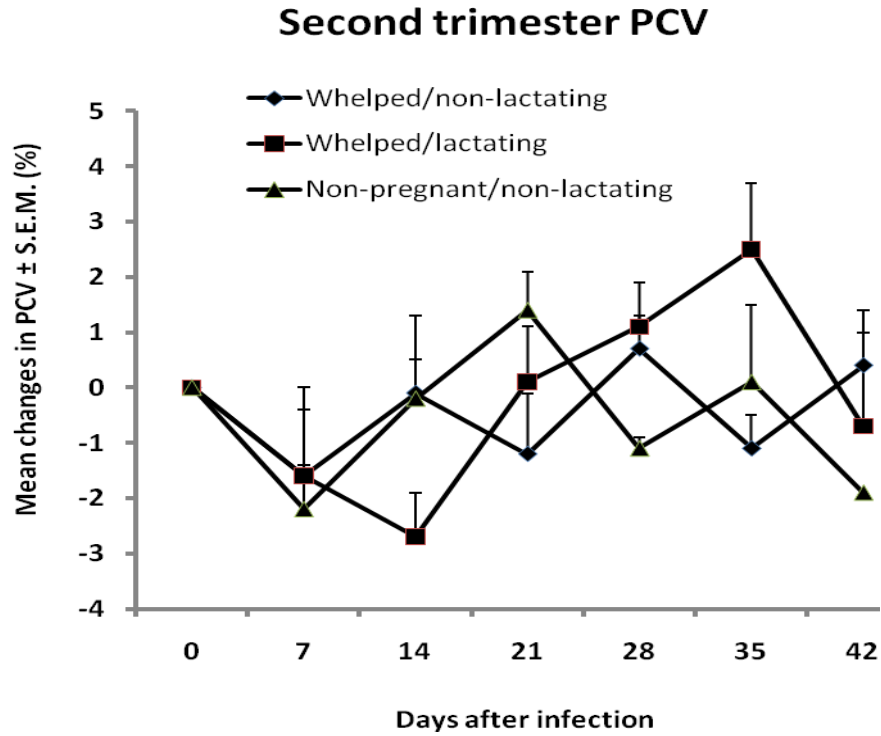
First trimester PCV

Figure 1e. Mean changes in PCV of lactating and non-lactating mice infected with 100 L3 *H. bakeri* in the first trimester of pregnancy.

to an increase in FEC (Crofton, 1958). However, the FECs were not higher in the periparturient period compared to other periods. This finding contrasts with the report that lactation in rats depressed the immune response to *N. braziliensis* infection in rats (Connan, 1970, 1972) shown by the persistence of the nematode. Available data on periparturient relaxation of immunity during GI nematode infections in goats are however, conflicting as reviewed by Chartier et al. (1998). For example, a study on fibre (Angora) goats showed a positive association of FEC with prolactin concentration around parturition whereas studies dealing with dairy goats, gave contrasting results (Chartier et al., 1998). However, Chartier et al. (1998) also reported that the mean FEC in pregnant goats were significantly higher during the two weeks before and two weeks after parturition compared with non-pregnant lactating animals. No rise in FEC was noticed in the mice in the present study either just before parturition or after parturition, nor was there a rise in FEC of the lactating mice compared to their non lactating counterparts, though WBs of lactating

mice were slightly higher. In the study of intestinal nematodes during lactation, no difference was also found to exist between the lactating and non-lactating mice (Deborah, 2002). However, Van Geldrop and Schillhorn van Veen (1976) had reported PPR in FEC in Udah ewes in the Zaria area in Nigeria. They were able to illustrate an increase in trichostrongylid egg output of the seven lambing and seven barren ewes used for the study but indicated that the increase reached a considerably higher peak in the lambing ewes. The highest FECs were recorded two weeks following lambing.

Periparturient rise in FEC may not be a common phenomenon to the Nigerian strain of outbred albino mice, or it may only occur at a particular period of the year. This study was conducted in Nsukka and lasted from September to October (late rainy season). A rise in FEC in ewes was first observed in Spring (Taylor, 1935), and this earned the phenomenon its synonymous name 'Spring rise.' Cvetkovic et al. (1971) had talked of the amplifying effects of parturition and lactation on an on-going 'Spring rise' in FEC where maximum post paturient

Second trimester PCV

Figure 1f. Mean changes in PCV of lactating and non-lactating mice infected with 100 L3 *Heligmosomoides bakeri* in the second trimester of pregnancy.

Third trimester PCV
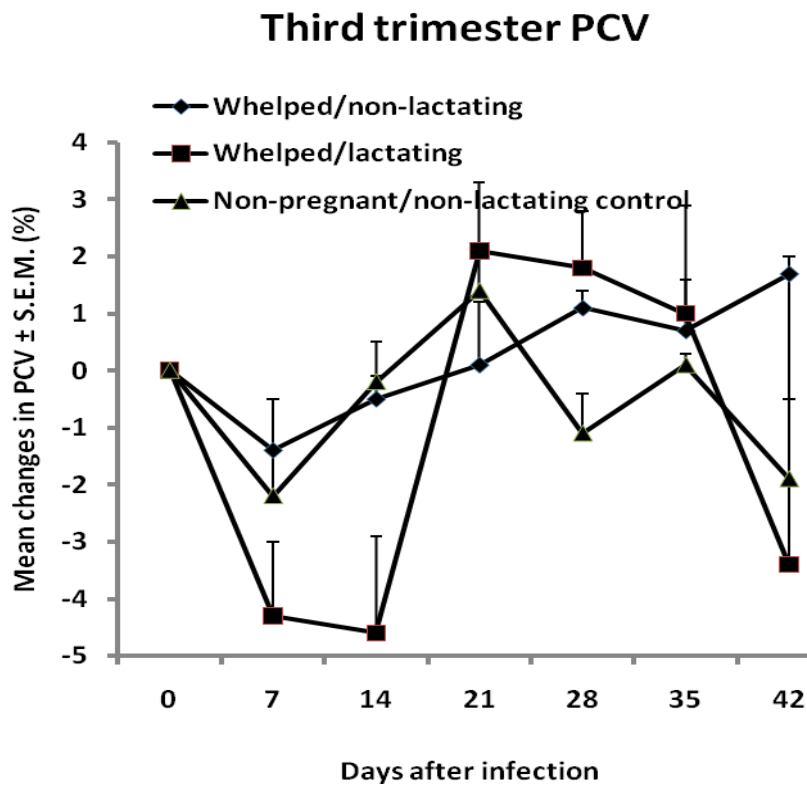
Figure 1g. Mean changes in PCV of lactating and non-lactating mice infected with 100 L3 *H. bakeri* in the third trimester of pregnancy.
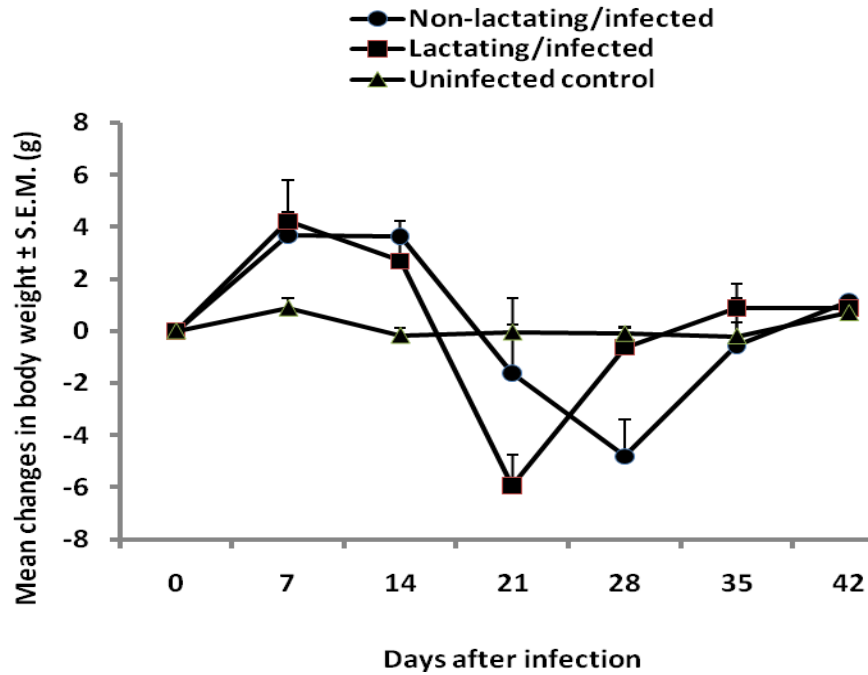
First trimester body weights

Figure 1h. Mean changes in body weight of lactating and non-lactating mice infected with 100 L3 *H. bakeri* in the first trimester of pregnancy.
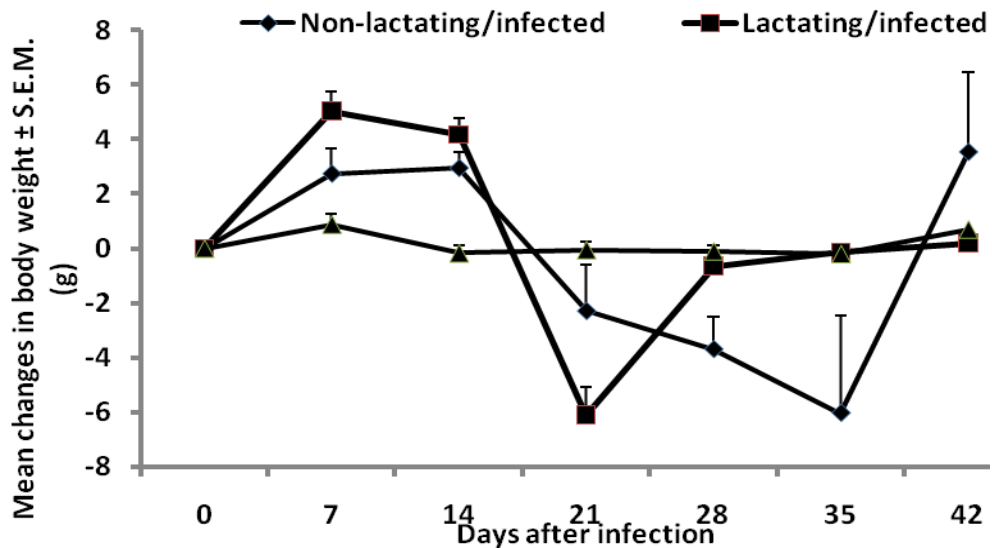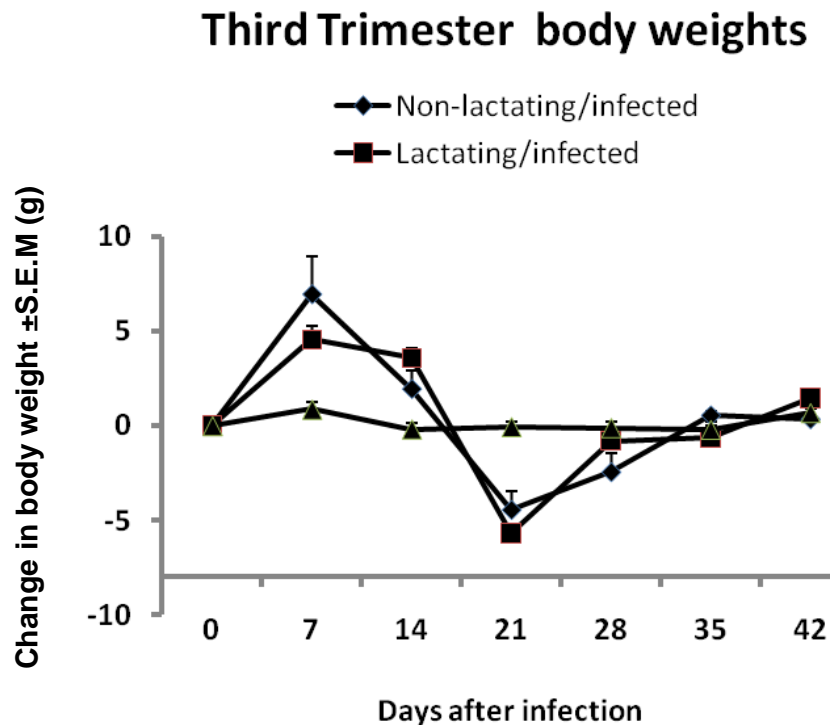
Second trimester bodyweights

Figure 1i. Mean changes in body weight of lactating and non-lactating mice infected with 100 L3 *Heligmosomoides bakeri* in the second trimester of pregnancy.

FEC were shown to occur only if lambing was timed to occur at the period during which a Spring rise was occurring in non-reproductive animals. It was explained that lambing period was not associate associated with a

Third Trimester body weights

Figure 1j. Mean changes in body weight of pregnant-lactating and pregnant-non lactating mice infected with 100 L3 *H. bakeri* in the third trimester of pregnancy.

periparturient rise in FEC. It has also been reported that 'Spring rise' occurs mainly but not restricted to female animals as some level of rise may occur in males and barren sheep (Crofton, 1954). Merino ewes were also monitored for a period of 21 months and no evidence suggestive of periparturient rise was observed (Allonby and Urquhart, 1975). PPR has not been demonstrated in Nigeria where *Ostertagia* spp. is non-existent and where *Haemonchus contortus* is the main cause of clinical helminthosis particularly in small ruminants (Van Geldrop et al., 1976).

A rise in FEC of Udah sheep in Zaria area of northern Nigeria was reported at the onset of the rainy season (van Geldrop and Schillhorn van Veen, 1976) in both lambing and non lambing ewes though the rise was considerably higher in the lambing ewes. Ogunsusi (1978) also reported such a rise but towards the end of the dry season. This might be suggestive that amidst prolactin's incrimination to be responsible for periparturient rise in ruminants, other factors may also contribute to the rise in FEC. Such factors may include season/time of the year, species and strain of the parasite, species and breed of the host, age of host, sex of host, nutritional status of the host, infection status of the host (whether concurrently infected with other parasites), and the environment of the host (altitude, weather). This is possible since some level of 'spring rise

has been reported in males and barren females (Crofton, 1954) while there may be little or no prolactin in such animals.

Conclusion

The intensity of infection with *H. bakeri* was greater in mice infected during the first trimester compared to those of mice infected during the second and third trimesters. However, there was no rise in FEC either just before parturition or just after parturition in lactating mice. Also, the WBs of lactating mice was just marginally greater than those of non-lactating mice. The findings in the present experiment suggest that *H. bakeri*-mouse model may not be ideal for the study of aspects of periparturient rise (PPR) in faecal egg counts in small ruminants.

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