

The Occurrence and Seasonal Magnitude of the Periparturient Rise in *Trichostrongylid* Nematode Egg Output in Breeding Yankasa Ewes in a Sub Humid Climate

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Abstract: Investigations were carried out on the occurrence of a periparturient rise in *Trichostrongyle* egg counts in breeding ewes grazing natural pastures. The first study using ewes in the last month of gestation and non-pregnant ewes as controls, showed that a significantly ($p < 0.05$) greater increase in egg counts (EPG) occurred in the lambing ewes when compared with the non-pregnant ewes. Peak EPG was 357.1 ± 94 four weeks after lambing while it was 190 ± 162 in non pregnant ewes. A second study using ewes grazing a separate pasture showed an exponential increase in mean EPG from 187 ± 124 pre lambing to 1589 ± 429 two weeks post lambing. A third study investigated the effects of season on the magnitude of the periparturient rise. Ewes whose lambings were timed to coincide with four seasons (late dry, early wet, late wet and late dry seasons) were monitored from 4 weeks pre to 5 weeks post lambing. The periparturient rise occurred irrespective of season of lambing but the magnitude was influenced by the season. The highest periparturient rise occurred in the late wet season (peak of 4201 ± 1813) four weeks post lambing while the lowest occurred when lambing coincided with the late dry season (peak of 1800 ± 765). Packed cell volume values in lambing ewes declined with increased egg counts but total protein values did not show appreciable changes. The periparturient rise in helminth egg counts is an important epizootiologic consideration in the study area and tactical treatment in its elimination will reduce infection for lambs, lower mortality and increase productivity of ewes.

Keywords: Egg count, periparturient rise, seasonal dynamics, sheep

INTRODUCTION

Gastrointestinal Nematodes (GINs) are a leading cause of clinical disease and death in grazing sheep worldwide, hindering both sheep production and profitability (Sutherland and Scott, 2010). Production losses are caused through reductions in nutrient availability to the host through both reductions in voluntary feed intake and/or reductions in the efficiency of absorbed nutrients as well as through increased nutrient loss (Edirisinghe and Tomkins, 1995). Lambs and ewes under productive stress are particularly vulnerable (Coop *et al.*, 2001). Pregnant ewes and does around the time of parturition are found to have increased Faecal Egg Counts (FEC), a phenomenon known as the periparturient rise (PPR) (Salisbury and Arundel, 1970).

Studies have shown that during late pregnancy and early lactation, systemic antibody levels and the cellular immune response become reduced (Huntley *et al.*, 2004). Subsequent to this, resistance to the establishment of incoming larvae, host control of egg

production of female worms and the capacity to expel mature worms may all be diminished (O'Sullivan and Donald, 1973; Donald *et al.*, 1982). The consequence of the parasitic load during the periparturient period has often been reported as the major source of pasture contamination in ruminants (O'Sullivan and Donald, 1973; Barger, 1993) and the first major source of larval challenge to young stock post weaning (Vlassoff, 1976). After weaning, the sheep's immunological response restores itself resulting in a rapid drop in FEC (Amarante *et al.*, 1992).

Some factors have been identified as modulators of the immune response during the periparturient period, like protein metabolism (Houdijk *et al.*, 2000; Donaldson *et al.*, 2001), host genetics and hormones such as prolactin (O'Sullivan and Donald, 1973). With protein metabolism It has been postulated that nutrients which would have been used by the immune system to fight parasites are diverted to reproductive functions; (e.g., foetal growth and milk production) (Houdijk *et al.*, 2000; Rocha *et al.*, 2011). Genetic variability and breed differences in the periparturient rise has also been

established in sheep and goats (Bishop and Stear, 2001; Chauhan *et al.*, 2003; Yadav *et al.*, 2006).

Several factors are known to determine the epidemiological patterns of the PPR. These include weather conditions, husbandry practices and type of helminth involved (Baker *et al.*, 1998; Rocha *et al.*, 2004). Therefore it is necessary to understand the epidemiology of helminth infections in animals of different breeds and climatic conditions so that targeted anthelmintic treatment can be provided for increasing productivity of the flock.

In Nigeria little information is available on the epidemiology of the PPR as a flock phenomenon. Van Geldorp and Schilhorn (1976) described its occurrence in a few Uda sheep while van Veen (1978) described it in 7 Uda sheep lambing over a one month period in the same area. The objective of this study was to determine the degree and seasonal variation of this phenomenon in lambing Yankasa ewes naturally infected with predominantly *H. contortus* infection in a sub-humid area of Nigeria.

MATERIALS AND METHODS

Study site: The National Animal Production Research Institute, (NAPRI) Shika, Zaria where these studies were carried out, is located on latitude 11° 12' N and longitude 7° 37' N with an altitude of 610 m and annual rainfall of 1107 mm. The climate of the area is sub humid while the vegetative zone is Northern Guinea savannah. Rainfall is distributed in such a way that the year can be divided into four seasons namely late dry (January-March); early wet (April-June); late wet (July-September) and early dry (October-December). The seasonal distribution of rainfall is about 0.1% during the late dry season; 25.8% in the early wet season; 69.6% in the late wet season; and 4.5% in the early dry season. The first significant rains come in late April or May and precipitation reaches a peak in July or August and ends abruptly in October but the pasture is sometimes still wet up to November. The average temperature and humidity during the wet season are 24.8°C and 72% respectively. The dry season is characterised by considerable diurnal variation in temperature with daily temperature ranging from 14-30°C with relative humidity of 10-20%. Fodder is generally scarce and of low quality in the late dry and early wet seasons. It is in abundant supply and of relatively good quality in the late wet and early dry season (Osinowo *et al.*, 1993).

Animals and management: Breeding of ewes was by flock or pen mating, to rams of the same breed. Following parturition, ewes and their lambs were separated into a nursing pen and kept under observation for one week after lambing. While indoors the ewes were given supplementary feed as well as chopped sun-

dried green fodder during the rainy season or hay during the dry season. All animals used for the experiment were naturally infected from pasture and did not receive any experimental infections. The periparturient period here was taken to include the period, four weeks before parturition and the immediate six weeks post parturition.

Experiment 1: Animals used for this experiment consisted of 36, 2-3 year old ewes, which were pregnant and were in the last stage of pregnancy. Twenty non-pregnant maiden ewes served as controls and grazed the same pasture with the pregnant ewes. The pregnant ewes lambed between the months of August and September but had inadvertently received a routine monthly anthelmintic treatment in July, a month before lambing was due to begin. Weekly rectal faecal samples were collected from each animal from four weeks pre to six weeks post lambing. The purpose of this experiment was to see if there was any difference in the helminth egg counts of ewes at lambing and that of non-pregnant ewes grazing the same pasture.

Experiment 2: These were also animals of the NAPRI sheep breeding project but these were housed differently and grazed a separate pasture from ewes in experiment one. They consisted of 35, 2-3 year old pregnant ewes in the late pregnancy and they lambed between the months of February and April. Weekly faecal samples were collected from these animals from 2 weeks before to 6 weeks after lambing. Weekly blood samples were also taken for Packed Cell Volume (PCV) Haemoglobin concentration (Hb) and Total Protein (TP) determination during the same period.

Experiment 3: Consisted of a total of 40 ewes selected from a flock of 63 ewes, 2-3 years old, which had been bought from the open market. They were housed and grazed separately from experiments one and two animals but feeding and management were the same. Four rams of the same breed selected from the NAPRI sheep project were joined to the ewes. The rams were fitted with aprons bearing coloured chalk so as to be able to identify any ewe that had come on heat and had been bred by the ram. The date on which any ewe was marked was taken and the ewe subsequently sampled weekly for faecal egg counts during the periparturient period. Ten ewes in late pregnancy were selected for each season. The seasons were late dry; early wet; late wet and early dry seasons. The ewes were monitored from 4 weeks pre to 5 weeks post lambing. Weekly blood samples were also taken for Packed Cell Volume (PCV) Haemoglobin concentration (Hb) and Total Protein (TP) determination during the same period.

Sample collection and analysis: Faecal samples were collected rectally from each animal at weekly intervals using disposable gloves. They were labelled and

transferred to a cooler with ice packs and kept refrigerated until analysis. Faecal Egg Counts (FECs) were performed according to standard parasitological methods (MAFF, 1977).

Larval culture: Faecal cultures for recovery of infective larvae were prepared by incubating 4-10 g faeces from pooled batches of faeces from the animals in lightly capped jars at 23-25°C in a humid atmosphere for 7 days. L3 larvae were then extracted using a Baermann funnel and larvae collected by draining the funnel into a petri dish. This was scanned using a stereo microscope for the presence of larvae and a sample transferred to a microscope slide. A drop of Gram's iodine was added to kill and stain the larvae. About 100 infective larvae from the culture were then differentiated at the generic level with the aid of standard methods (MAFF, 1997).

Blood collection and analysis: About 5 mls of blood were collected by jugular venipuncture weekly into labelled vacutainer tubes with Ethylene Diamine Tetracetic Acid (EDTA) as anticoagulant for haematological studies. The vacutainers were placed on ice in a cooler and transported to the laboratory.

The haematological indices monitored were haemoglobin, estimated as cyanmethaemoglobin using a Coulter Haemoglobinometer (Coulter Electronics Ltd, England). Percentage PCV was determined using a Hawksley microhaematocrit centrifuge and reader, the tubes being centrifuged for five minutes at 1200 g. Total protein was read using a Goldberg Refractometer.

Data analysis: The weekly strongle egg count for each animal was transformed, replacing the actual count, x , by $\log(x+1)$. The means of the transformed counts for the different groups in a given period were analysed by using one-way analysis of variance and the student's test. The major effect of the transformation was to stabilise the variance and compensate for the zero egg counts. After analysis, the egg count values were re-transformed for recording.

RESULTS

Experiment 1: Helminth egg counts in lambing and non-lambing ewes: In experiment 1, pregnant ewes produced significantly ($p<0.05$) more eggs during the periparturient period than did non-pregnant control ewes (Fig. 1) Faecal egg counts increased from a mean of 19.4 ± 11 pre-lambing to 357.1 ± 94 egg 4 weeks post lambing while in non-pregnant ewes equivalent egg counts at this time were 14.0 ± 12 and 190.0 ± 162 , respectively. Individual peak counts ranged from 600-

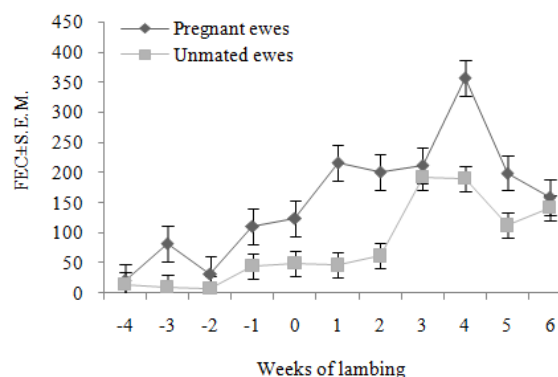


Fig. 1: Mean Faecal Egg Counts (FEC) in pregnant and maiden ewes grazing the same pasture monitored from 4 weeks before to 6 weeks after lambing. (The animals had inadvertently received a routine monthly anthelmintic treatment a month before lambing was due to begin)

2400. From 6 weeks post lambing there were no appreciable differences in helminth egg counts of lambing and non-lambing ewes.

Experiment 2: Egg counts and haematological indices of lambing ewes: A post parturient increase in egg output that had a peak 2 weeks post lambing occurred in the ewes on lambing (Fig. 2). At the peak of egg output at 2 weeks post lambing egg counts had increased from 187 ± 124 two weeks pre-lambing to 1589 ± 429 egg, a more than 8-fold increase over pre-lambing values. Egg counts were significantly higher ($p<0.05$) than pre-lambing values between the week 0 of lambing and 3 weeks post lambing PCV decreased with increasing egg counts, being lowest four weeks after lambing. Haemoglobin values followed the same trend, but Total protein values remained fairly stable.

Experiment 3: Helminth egg counts and haematology of ewes lambing in the different seasons: The mean periparturient egg counts of ewes lambing in the different seasons are shown in Fig. 3. Ewes that lambed in the early and late wet seasons as well as those lambing in the early dry season showed a rise in faecal egg counts post lambing while ewes lambing in the early dry season did not show a post lambing rise in egg counts but had significantly higher ($p<0.05$) egg counts pre-lambing, than ewes lambing at other seasons. The egg output pattern and magnitude varied in the various seasons and was minimal in ewes lambing during the late dry season. In ewes that lambed in the early dry season, egg counts progressively increased to a peak of 2860 ± 834 egg on the week before lambing and subsequently showed decreases. In ewes lambing in the early wet season egg counts progressively increased week 1 after lambing to a peak of 3838 ± 598 on week 5 post lambing. Ewes that

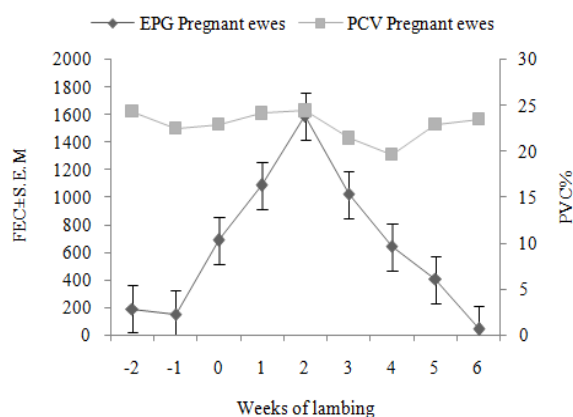


Fig. 2: Mean Faecal Egg Counts (FEC) and Packed Cell Volume (PCV) in bred ewes on pasture in the dry season months monitored from 2 weeks before to 6 weeks after lambing

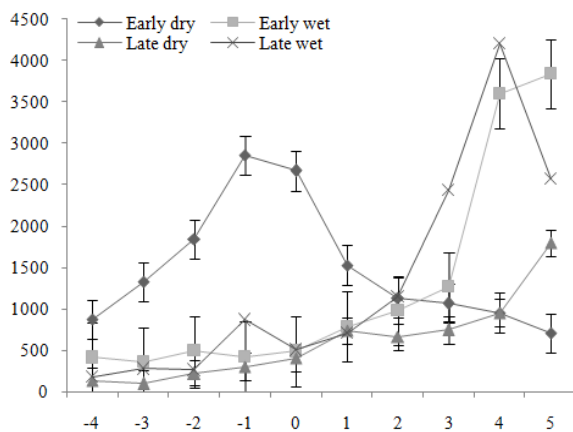


Fig. 3: Mean Faecal Egg Counts (FEC) of ewes on pasture that lambbed in the early dry, early wet late dry and late wet seasons respectively, monitored from 4 weeks before to 5 weeks after lambing

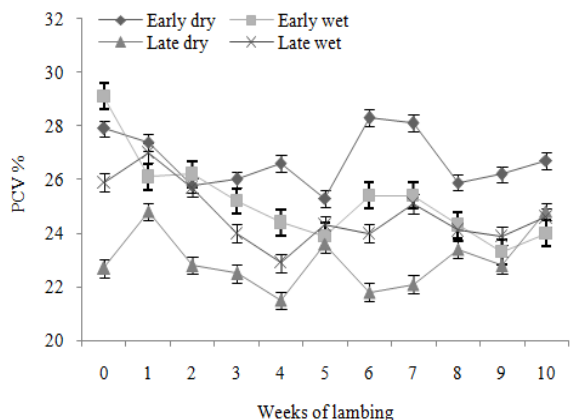


Fig. 4: Mean Packed Cell Volume (PCV) values of ewes on pasture that lambbed in the early dry, early wet late dry and late wet seasons, respectively, monitored from one week before to 10 weeks after lambing

lambled in the late dry and late wet seasons also showed progressive increases in helminth egg counts after lambing but attained peaks of 1800 ± 765 at week 5 and 4201 ± 1813 at week 4 post lambing, respectively. Larval culture showed that *Haemonchus contortus* was the main contributor to the rise in helminth egg counts.

Post lambing, ewes that lambbed in the early dry season had PCV that were consistently higher ($p < 0.05$) than PCV of ewes lambing in the early wet and late dry seasons (Fig. 4). Ewes that lambbed in the late dry seasons had consistently lower PCV than ewes that lambbed at any other season. PCV of individual ewes ranged from 16-32 gm/dL and anaemic ewes showed pallor of the mucus membrane as well as emaciation.

Moreover, there were no significant differences in helminth egg counts in the ewes in early and mid pregnancy (not shown) until a few weeks before lambing, suggesting that the increased egg counts were associated with endocrine events at the periparturient period.

DISCUSSION

A rise in Faecal Egg Count (FEC) occurred during the periparturient period in lambing ewes in the three experiments. In contrast, no appreciable increase in egg counts occurred in non-lambing and in pregnant ewes, thus demonstrating that increase in faecal egg counts was associated with parturition and lactation. This finding is in agreement with the report of (Almeida *et al.*, 2012; Gibbs, 1986; O'Sullivan and Donald, 1973). A relaxation in the immune response to helminth infection has been demonstrated to occur during late pregnancy and early lactation (Jackson *et al.*, 1988; McAnulty, 1990) and this occurrence may be responsible for the substantial faecal -egg count rise of ewes during the periparturient period. The PPR in egg count rose to a peak from between one week before to 5 weeks post lambing and this is similar to the report of two weeks before lambing to six or more weeks after lambing, reported by Maizels *et al.* (2012). The rise in egg count did not start as far back as 6 weeks pre parturition as reported by Jackson *et al.*, (1988) and Rocha *et al.*, (2011). The rise in egg counts reached a peak rapidly before starting a decline between 2 and 4 weeks after lambing. Such an early peak in egg counts is associated with helminths with short generation intervals such as *Haemonchus*. The time of peak egg output varies with the species present (Borgsteede, 1978; Chauhan *et al.*, 2003) and so the early peak of 2-4 weeks in the faecal egg counts post lambing, reflects the short generation interval of 14-18 days for *H. contortus* which was the predominant species present in the ewes. *Haemonchus* is able to take the advantage of favourable environmental conditions as in a sub humid climate within a short time as has also been reported by Tembely *et al.* (1998); Ng'ang'a *et al.* (2004). The predominance of *Haemonchus* is a threat to profitable sheep production in the area given that this parasite can

cause severe anemia and hypoproteinemia, depression, loss of body condition, reduced productivity and possibly death (Amarante, 2011).

The decline in egg counts observed after the peak values may be a self-cure reaction. Such decrease in egg counts are known to be as a result of host immune reaction against infection (Beasley *et al.*, 2010) and can be taken as criteria for a return of immunity after the periparturient immunosuppression. This study agrees with the theory of a 'window effect' as reported by Jackson *et al.*, 1988. The authors suggested that a narrow period of marked immune depression occurred at/or around parturition and on either side of this the immune response gradually decreases prior to and increases after parturition. Therefore any larval infection which develops before or after this period, encounters a more responsive immune system albeit still impaired as compared to that of a resistant animal (Jackson *et al.*, 1988), but capable of suppressing egg production and reducing establishment of larvae. This could explain the findings of this study in which ewes on infected pasture were able to show low egg counts until shortly before parturition but this ability was lost close to parturition and the few weeks following parturition. Not long after parturition (2-5 weeks in this study) resistance of the animals was restored. Some authors have reported that ewes may be in the immune suppressed state up to 6 but not later than 12 weeks post lambing (McAnuit, 1990; Amarante *et al.*, 1992).

The same pattern was seen in wet and dry season lambing ewes though the magnitude of the rise differed with the season, with ewes lambing in the rainy seasons showing greater intensity of infection and more animals manifesting poor clinical condition as a result of infection. These findings are similar to that observed in other breeds of sheep (Vercruysse, 1983; Ng'ang'a *et al.*, 2004). The greatest increase in EPG of up to 22% over pre lambing values occurred in the late wet season which is in consonant with climatic conditions which favour larval availability on pasture at this time. There was also a 9% increase in egg count over pre lambing values in the early wet season. However, increase of 13% over pre lambing values were not expected in ewes that lambed in the late dry season. Although pasture larval counts were not done in this study, earlier reports by Ogunsusi (1979a); Fabiyi *et al.* (1979) in the same northern Guinea savannah area indicated that infective larvae were not available on pasture in the months of the late dry season. A seasonal arrest of development of *H. contortus* of sheep in the Zaria area is known to occur as the dry season sets in, being greatest among larvae ingested from pasture in the late dry season (Ogunsusi, 1979b). These larvae remain arrested until early wet season when they mature asynchronously. The occurrence of the PPR in ewes that lambed in the late dry season, when there is little larvae on pasture, is likely to be related to the maturation of some larvae previously arrested in the

ewes. Maturation of arrested larvae during the dry season in the same area has been associated with outbreaks of dry season clinical parasitic gastroenteritis (van Veen, 1978; Fabiyi *et al.*, 1979). The lowered immune state of the periparturient ewes, might have stimulated the re-activation of some of the arrested larvae as well as allowed an increase in the fecundity of the residual adult population that had low egg productivity during pregnancy. Michel (1976) also showed a relationship between the physiological status of the ewe and the "activation" of arrested larvae acquired the previous grazing season. He proposed that parasite populations are controlled by a biorhythm of parasite origin, in that arrested larvae in sheep of all descriptions mature at the same time, while in parturient and lactating animals, parasites are retained longer than in barren animals. The parasitological findings of this study are in agreement with this explanation.

The periparturient period comprising late gestation and lactation are the reproductive stages in which ewes suffered most from parasitism by gastrointestinal nematodes. The intensity is influenced by season of lambing and the occurrence of hypobiosis of helminth spp that undergo it in the area. Breeding ewes would require increased care and management at this phase of increased susceptibility to parasitism. This should include health and nutritional management.

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