Pakistan J. Zool., vol. 41(6), pp. 437-443, 2009.

Peri-Parturient Rise in Faecal Nematode Egg Counts with Reference to *Haemonchus contortus* in Bulkhi Ewes in Northern Punjab, Pakistan

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Abstract.- The occurrence of the peri-parturient rise (PPR) in fecal egg count (FEC) phenomena in Bulkhi ewes and its subsequent impact on naive lambs reared in a traditional semi-intensive husbandry system were monitored at the Small Ruminants Research Station, National Agricultural Research Centre, Islamabad, Pakistan. In this study, two ewe groups, pregnant/lactating (n=37) and open/non-pregnant (n=37), were observed for the PPR phenomena in the sub-tropical area of Pakistan. A significant difference ($P< 0.01$) was noted for FEC, individual larval culture, packed cell volume (PCV) and haemoglobin (Hb) level in the pregnant/lactating ewes as compared to the open ewes throughout the study. Faecal examination showed consistently higher, predominantly *Haemonchus contortus,* FEC with lower PCV and Hb level in the pregnant/lactating ewes. The sharp increase in FEC occurred two weeks before lambing and persisted for 12 weeks after lambing. The results showed that the PPR in FEC was associated with both gestation and lactation which provided a large number of third-stage infective larvae (L_3) on pasture. These larvae were considered the primary source of infection for the lambs. Thus, infection in lambs showed an initial rise in FEC after four weeks when highly susceptible lambs were allowed to graze along with their dams on the same naturally contaminated pastures. A control measure to consider would be to deworm ewes before lambing or in early lactation to reduce pasture contamination and infection of lambs.

Key words: Bulkhi ewes, fecal nematode eggs, *Haemonchus contortus*, lactation, naive lambs.

INTRODUCTION

There is substantial evidence that the peri-parturient rise (PPR) in fecal egg count is of great importance in the epidemiology of gastrointestinal nematodes and has often been considered as a major source of pasture larval contamination in small ruminants (Romjali *et al*., 1997; Tembely *et al*., 1998; Keyyu *et al*., 2001; Ng'ang'a *et al*., 2004; Mandonnet *et al*., 2005). The strong evidence that the PPR is a consequence of immunosuppression associated with lactation which allows a dramatic increase in faecal egg output from just prior to parturition and during lactation has been documented world wide (Zajac *et al*., 1988; Agyei *et al.*, 1991; Singh *et al*., 1997; Keyyu *et al*., 2001). This temporary loss of immunity may result in resumption in development of hypobiotic larvae, enhanced fecundity of adult female worms, the acquisition of new infections and possibly the failure to eliminate some existing worms (Coop *et al*., 1990; Tembely *et al*., 1998; Waller *et al*., 2004). The ultimate result of the PPR is an increased number of infective larvae (L_3) on pasture that become an important source of infection to lambs when they begin to graze (Donald *et al*., 1982; Eugene *et al*., 1992). With reference to Pakistan, no information is available regarding the role of the PPR on the biology of *Haemonchus contortus*. The objective of this study was to determine the degree and duration of this phenomenon in lambing and non-lambing ewes naturally infected with *H. contortus* infection in the sub-tropical area of Pakistan. The results can then be taken under consideration to plan strategic deworming programs along with husbandry management practices during

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0030-9923/2009/0006-0437 $ 8.00/0

the peri-parturient period to keep the infection in check especially in lambs in this region.

MATERIALS AND METHODS

Study site and experimental animals

The study was conducted at Small Ruminants Research Station, National Agricultural Research Centre, Islamabad, Pakistan. The sheep breed used was Bulkhi commonly known as Afghani sheep. This breed is mostly reared by nomadic tribes and is well adapted to the moderate climate of the sub tropics. They are light brown in colour and have a fat tail. They are maintained for mutton and coarse wool. Lambing interval is eight to ten months and they usually have single lambs.

Ewes management

Ewes were managed in a semi-intensive system, where they grazed during the day from 0800 to 1700 hours. They grazed areas of naturally growing grasses, regenerating shrubs and herbs. At night, they were housed in brick sheds in concrete-floored pens. Animals were fed on PARC Feed Technology Brand pelleted diet (40% cotton seed cake, 20% maize grains, 16% wheat bran, 10% rice polishing, 14% cane molasses, and 1% mineral mixture) supplemented throughout with a diet of pelleted concentrate at the rate of 0.54/ kg/animal/day. The composition of diet was 15% crude protein and 75% total digestible nutrients on dry wt. basis. Green fodder and water were provided *ad libitum* during day and night.

Breeding

Ewes and rams were maintained separately throughout the year except during breeding season which was in the fall, 2004. Lambing occurred in the spring, 2005.

Lambs management

Lambs were kept on their mother's milk with supplementary feed and were allowed to accompany their dams to pasture as soon as they were able to walk.

Study design

Two hundred ewes were bred to ten rams in September and October, 2004. After 35-45 days, the ewes were examined by an ultrasound machine to determine conception. Thirty-seven pregnant and 37 open/non-pregnant ewes were selected and each group was of comparable age and body weight. Lambing commenced in the second, third and fourth week of February, 2005. Intensity of *Haemonchus contortus* infection was monitored from two months prior to lambing (December & January) uptill four months after lambing (March-June) in both ewe's groups.

Sampling schedules

Ewes

Fecal and blood sampling started in December, 2004 on a fortnightly basis. From January through April, 2005 sampling was on a weekly basis. Subsequently, sampling returned to a fortnightly basis through June.

Lambs

The total number of ewes was 39 in each group (open and pregnant), from which 47 lambs were born in the month of February. Out of 39 ewes, 37 (open and pregnant) and out of 47 lambs, 42 were considered throughout the study, as 2 pregnant ewes and 3 lambs were dewormed while 2 lambs died during the study period. Thirty seven lambs, selected for the study, were maintained by nursing as well as supplement feed for about one month. These lambs were allowed to graze on the highly contaminated pasture along with the permanent flock. After two weeks of grazing, the lambs were sampled on a fortnightly basis from April, 2005 through October, 2005 to determine the level of *H. contortus* infection**.**

Parasitological measures

Faecal collection

Faecal egg counts were conducted using the modified McMaster Method (Coles *et al*., 1992) with saturated sodium chloride as the flotation fluid. A sensitivity of 50 eggs per gram (epg) of faeces was used. Faeces were collected from all ewes (non-pregnant and pregnant) for 11 months and from lambs for six months.

Coprological culture

The larvae were harvested from faecal culture from studied ewes and lambs by Baermannization process (MAFF, 1997). The identification of third-stage infective larvae (L_3) was carried out by established keys and descriptions (MAFF, 1997).

Haematological parameters

Blood samples were taken from the jugular vein into 5ml ethylene diamine tetra acetic acid (EDTA) coated vacutainer tubes. Packed cell volume (PCV) and haemoglobin (Hb) level were determined as described by Torres-Acosta *et al*. (2004).

Statistical analysis

Data was analyzed, by statistical package POST HOC TEST (univariate analysis of variance) was performed. Faecal egg count and larval culture data were transformed before analysis [log10 (n+1)] to stabilize the variance.

RESULTS

Faecal egg count in ewes and lambs

At the start of the study, both pregnant/lactating and open/non-pregnant ewes had almost similar faecal egg count (FEC). A month before lambing and during the lambing period, the FEC started to increase considerably in the pregnant/lactating and mildly in open ewes. During the lambing (February) and post-lambing periods (March-June), FEC was significantly ($P<0.05$) higher for pregnant/lactating ewes than for open ewes. Higher FEC was recorded during the post-lambing as compared to the lambing and pre-lambing periods (December and January). In the pregnant/lactating ewes FEC peaked in March (4092.5±105.3) and the open ewes FEC peaked in April (2028.57±67.3). While in lambs FEC steadily increased from April and peaked in October (4514.28±108.4) as shown in Figure 1.

Fecal culture in ewes and lambs

The number of L_3 recovered from fecal culture followed the same pattern as the FEC with a significant ($P<0.05$) positive correlation. Higher individual larval count was recorded during the post-lambing as compared to the lambing and pre-lambing periods. The pregnant/lactating ewes fecal culture peaked in March (3154.82±84.4) and the open ewes fecal culture peaked in April (1569.14±64.9). Lambs faecal culture steadily increased from April and peaked in October (2301.29±69.8).

Moreover, the frequencies of generic composition of trichostrongyles larvae (L_3) from individual faecal cultures showed that *H. contortus* is the predominant specie followed by *Trichostrongylus* spp and *Oesophagostomum columbianum* throughout the study period (Table I).

Packed cell volume and haemoglobin levels in ewes and lambs

At the start of the study, both pregnant/ lactating and open ewes had almost similar packed cell volume (PCV) and haemoglobin (Hb) levels. The PCV and Hb decreased for both groups and the levels for the pregnant/lactating ewes were significantly ($P<0.05$) lower than the open ewes throughout the study. The highest level of PCV and Hb for pregnant/lactating ewes (20.79±0.9 and 16.57±0.7) and open (21.99±0.9 and 17.38±0.7) ewes was observed in the month of December. The lowest level of PCV and Hb for pregnant/lactating ewes (8.71±0.4 and 6.73±0.4) and open ewes (15.21±0.6 and 10.93±0.5) was observed in March and April, respectively. Lambs PCV and Hb steadily decreased from April and was the lowest in October (8.65±0.4 and 6.67±0.3) as shown in Figure 2. For both ewes and lambs, the PCV had a significant ($P<0.05$) positive correlation with Hb level and a significant ($P<0.05$) negative correlation with FEC and faecal culture.

DISCUSSION

The results of this study showed that faecal egg count (FEC) in pregnant/lactating Bulkhi ewes increased faster and maintained consistently greater level than open/non-pregnant ewes while grazing the same pasture. By shedding a greater number of nematode eggs during this period, pasture contamination would be increased and infectivity to new born lambs would be assured. The peri-parturient rise (PPR) phenomenon was clearly observed in pregnant/lactating ewes; however, a similar but much reduced pattern was observed in

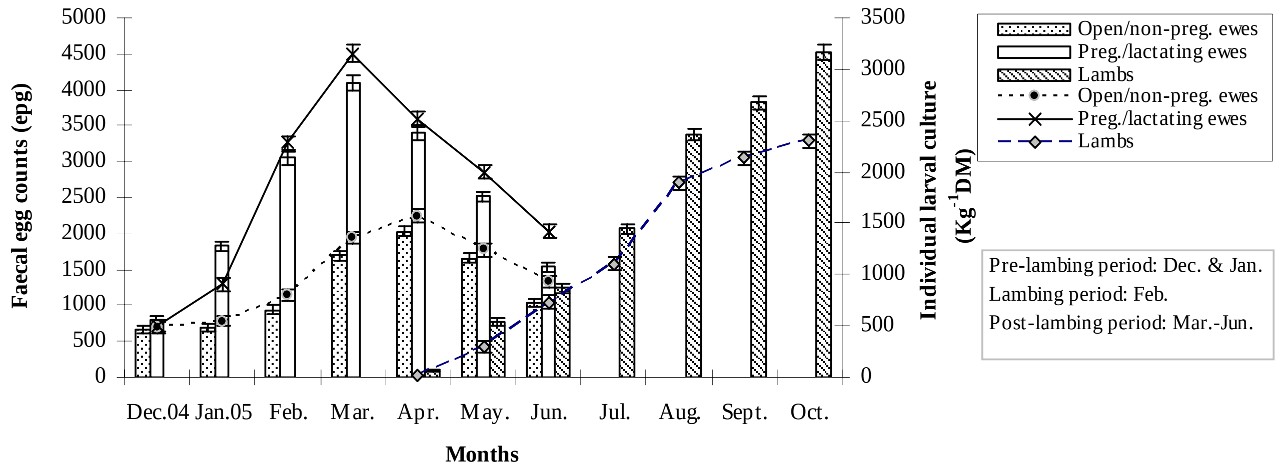

Fig. 1. Pattern of the peri-parturient rise in the faecal egg counts (bars) along with individual larval culture (lines) in two ewe's groups and lambs.

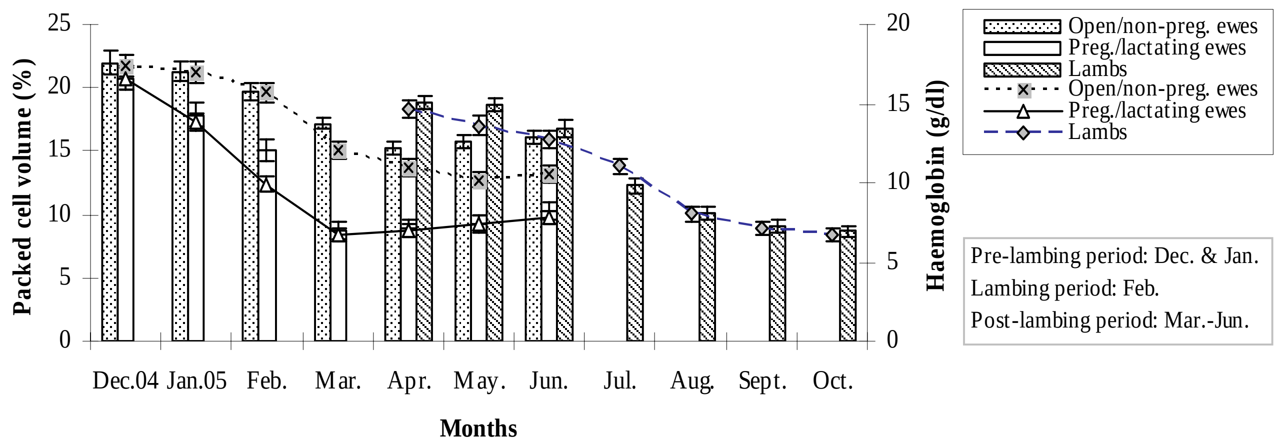

Fig. 2. Effect of peri-parturient on the packed cell volume (bars) and haemoglobin concentration (lines) in two ewe's groups and lambs.

Table 1.- Generic composition (Mean±SEM) of trichostrongyles larvae (L_3) recovered from faecal cultures in two ewe's groups and lambs.

Parasites	Open/non-preg. ewes (December – June)	Preg./lactating ewes (December - June)	Lambs (April-October)
Haemonchus contortus	314.28±15.4^{a*}	407.14±18.1^{a}	428.57±19.0^{a}
Trichostrongylus colubriformis	142.85±6.3^{b}	78.57±3.9^{c}	100.01±4.29^{b}
Trichostrongylus axei	100.01±4.29^{c}	85.71±4.1^{c}	57.14±2.8^{c}
Oesophagostomun columbianum	157.14±7.8^{b}	142.85±6.3^{b}	128.57±4.6^{b}

*Mean±SEM. Values with different superscripts in a column differ significantly (P< 0.05).

the open ewes. This has already been previously reported in other regions of the world (Lyons *et al*., 1992; Rahman and Collins, 1992; Chartier *et al*., 1998; Baker *et al*., 1998; Ng'ang'a *et al.*, 2004; Mandonnet *et al*., 2005).

Based on fecal culture, *Haemonchus contortus* is the predominant contributor to the PPR in this sub-tropical area of Potohar region, Pakistan. In support of this, the packed cell volume (PCV) and haemoglobin (Hb) levels decreased for both ewe's groups with the pregnant/lactating ewes being consistently lower through-out the study period which indicated a heavier infection. These results are consistent with the contention that the PPR is due to continuously acquired infection from pasture as the environmental conditions are found to be conducive for the rapid development of free-living stages of *H. contortus*. Therefore, pregnant/lactating ewes showed a much more distinct PPR in FEC as compared to open ewes. This trend is accordance with the findings of others (Yazwinski and Featherstone, 1979; Coop *et al*., 1990; Singh *et al*., 1997). There are a number of reasons which may be considered for the onset of this phenomenon. These include a breakdown in immunological responsiveness to parasitic infection during late gestation and lactation (Fleming, 1993; Dorney *et al*., 1995; Kahn *et al*., 2003) and resumption of development of arrested larvae within the host (Blitz and Gibbs, 1972; Armour, 1980). This results in female worms laying more eggs, an increased number of adult worms and decreased ability to expel adult worms (Donald *et al*., 1982; Michael, 1991; Coyne *et al*., 1991).

The results of the present study revealed that *H. contortus* was the most ubiquitous and predominated gastrointestinal parasite in Bulkhi ewes followed by *Trichostrongylus* spp. and *Oesophagostomum columbianum*. The higher incidence of infective larvae (L_3) of *H. contortus* could be due to the fact that this nematode has a relatively short generation interval (Grant, 1981) as more prolific egg producer (Hunter and Heath, 1984) and its ability to take the advantage of favourable environmental conditions prevalent in tropical and sub-tropical region as reported by (Tembely *et al.*, 1998; Ng'ang'a *et al*., 2004; Chaudhry *et al*., 2007). Moderate occurrence of *Trichostrongylus* spp. and low frequency of *Oesophagostomum columbianum* as compared to *H. contortus* during PPR phenomena could be due to low resistance of free-living stages of these parasites to prevailing weather conditions in the studied area.

There have been reports that over-wintering infective larvae are of little significance for spring-born lambs compared with larvae arising from the PPR in FEC (Salisbury and Arundel, 1970; Donnelly *et al*., 1972). However, it is generally believed that arrested development takes place in temperate regions where extreme cold conditions may be responsible for the onset of PPR (Michel, 1974; Armour and Bruce, 1974). It may be inferred from the present study that *H. contortus* exists throughout the year with a contribution of continuous infection to small ruminants which is in agreement with Tembely *et al*. (1998) and Mandonnet *et al*. (2005) that several abiotic factors (rainfall, temperature, moisture, sunshine intensity) and biotic factors (feeding condition during pregnancy, stocking rate and inter and intra specific interaction in parasites) are major reasons for higher incidence of infective larvae on the pasture.

Increased egg production is the defining characteristic of the PPR which was observed from two weeks before lambing and persisted for 12 weeks in lactating ewes after the commencement of lambing. These observations are in agreement with the findings of Tembely *et al*. (1988) and Baker *et al*. (1998). It has also been observed that the PPR has a substantial affect on new born lambs as the infection rate increases many fold in these young animals.

Results showed that pregnant/lactating ewes played a key role during the peri-parturient period to contaminate the pasture to a great extent which in turn provided a massive infective larval population, predominantly *H. contortus*, for ingestion by grazing lambs. This is in accordance with the findings of others (Michel, 1974; Herd *et al*., 1983; Eugene *et al*., 1992). The consequence of such infection is deleterious to lambs with prominent signs of haemonchosis, *i.e.* reduction in PCV and Hb levels. The intensity of the PPR in FEC may have direct and indirect consequences on ewes and their lambs regarding their health and productivity. Thus, adequate control of ewe infection rate may lead to substantial improvements in herd productivity after lambing and weaning, particularly in those flocks which are maintained under sub-tropical climatic conditions where nematode infections are common. With the focus to reduce the PPR intensity under sub-humid conditions, it is recommended for lambs to be included in the anthelmintic treatment programme especially after weaning. Infection of lambs at a very young age needs special attention because of their greater susceptibility to the effect of infection at this age. It can be concluded that pregnant/lactating ewes are an important source of gastrointestinal nematode infection, particularly with *H. contortus*, for new born lambs. Thus, a timely anthelmintic treatment before and after lambing may be advised for ewes.

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(Received 20 September 2008, revised 8 June 2009)