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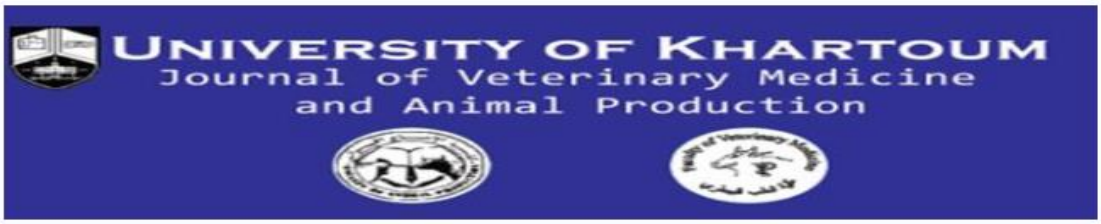

Epidemiology and seasonal dynamics of internal parasite infections in small ruminants at Ukulinga Research Farm, South Africa

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Abstract
An epidemiological study of internal parasite infections of ewes and does was carried out at the University of KwaZulu-Natal, Ukulinga Research Farm in South Africa for 1 year. The experimental animals consisted of a total of 16 animals, including 8 Merino sheep ewes and 8 Ngunigoats does. These animals grazed on kikuyu pasture together with a larger university flock. Parasitological data of nematode egg counts (EPG), infective larvae (L3) from faecal culture and infective larvae found in the pastures were recorded monthly. Coccidian oocysts and live weight were also recorded. The measured parameters showed distinct seasonal effects. The highest level of infections occurred in December and the lowest level of infections occurred in July. In the faeces of the experimental animals *Trichostrongylus* species were the most prevalent parasites, followed by *Strongyloides* species, *Haemonchus contortus*, *Nematodirus* species, *Cooperia* species, and on *Eimeria* species. Seasonal effect was significant ($P<0.05$) on EPG, the infective larvae of the faecal cultures, pasture L_3 stages and *Eimeria* oocysts counts. Similarities were observed in EPG and live weight gain during the year. Information obtained gave base data for suggesting a control program with a minimum anthelmintic use.

Keywords: parasites, nematode, coccidian oocysts, sheep, goats, egg counts

Introduction

At high level *Eimeria* species and gastrointestinal nematodes can kill small ruminants (Agyei *et al.*, 2004). In addition they cause lower productively due to poor growth and body weight gain (Tembely *et al.*, 1997 and Abebe *et al.*, 2007). Understanding the parasite epidemiology and the factors that affect parasite growth are crucial for controlling internal parasites (Tembely *et al.*, 1997; Waller, and Thamsborg, 2004 and Sissay *et al.*, 2007). Moreover, seasonal dynamics affect parasite growth and development (Teel *et al.*, 1996 and Abebe *et al.*, 2007). The seasonal dynamics of nematode infection are the consequence of complex inter-relationships between sheep, their husbandry and the prevailing climate Vlassoff *et al.*, 2001 and Gebeyehu *et al.*, 2013).

The patterns of pasture contamination by nematode eggs, the larvae and the levels of infection in ewes and does are similar (Sissay *et al.*, 2007). The degree of pasture contamination is determined by the number of infective larvae on the pasture (Hale, 2006). However, many factors such as climatic conditions, the deposition of helminth eggs in faeces and the subsequent development of the parasites as well as the effect of birds, insects, fungi and wild mammals influence the development, survival, distribution and migratory behavior of the free-living larvae seen on pastures (Stromberg, 1997). These factors are highly changeable according to the season. Faizal *et al.* (1999) conducted an experiment to assess the combined effect of *Eimeria* species and gastrointestinal nematode infections on goats, in order to develop appropriate control strategies for parasitic infections.

The present study was carried out to determine the epidemiology of *Eimeria* species and gastrointestinal nematode parasites and their effect on small ruminants. The effect of seasonal dynamics on transmission and development of internal parasites was also examined.

Material and Methods

Study area

This study was conducted in the Livestock Section, of the University of KwaZulu-Natal Research Farm at Ukulingajust outside Pietermaritzburg in KwaZulu-Natal province in subtropical hinterland which is approximately 700 m above sea level. The climate is characterized by annual rainfall of 735mm, which falls mostly in summer between October and April. The maximum and minimum mean annual temperatures are 25.7 and 8.9°C, respectively. Light to moderate frost occurs occasionally in winter.

Experimental animals and animal management

Small ruminants (8 sheep ewes and 8 goat does), aged 7-18 months with an initial weight of 39 ± 7.6kg were used from February 2008 to January 2009. The weight of the animals was taken monthly. During the experimental period the animals were allowed to graze freely on contaminated kikuyu pasture which was divided into four paddocks. Water was available *ad libitum* in troughs. Experimental animals were allowed to graze together with other animals on the farm and were managed similarly to the farm flock during the one year study. No supplemental feed was given. Anthelmintics were given eleven times during the study period as indicated in Figure 2. Hoof trimming occurred every six months and the offspring were tagged when necessary.

Sample collection and parasitological analysis

Faecal samples were taken monthly, from the rectum of the experimental animals, and placed in plastic bags bearing the animal's identification number. Samples were then conveyed to the Animal and Poultry Science Departmental laboratory where the parasitological assays were done.

Faecal nematode egg counts and count of coccidian oocysts were done using the McMaster Technique (Hansen and Perry, 1994). Enumeration of faecal eggs and coccidian oocysts were usually completed on the day following the collection day. Fifty six ml of saturated salt solution was added to four grams of faeces in a beaker. The faecal suspension was filtered into another beaker, and the sub samples were examined under a light microscope at 100 magnifications after both wells of the McMaster counting chamber were filled with the suspension and were allowed to stand for 5 minutes. The number of nematode eggs and coccidian oocysts in both wells of the McMaster chamber was multiplied by 50 to get EPG and OPG.

The faeces were also pooled and mixed per group, sub-sampled into 6 trays and incubated for 15 days at 27 °C. Samples were kept damp by watering every day at 10:00 h during the period of incubation. The Baermann technique was then used to identify infective larvae according to Hansen and Perry (1994). Nine grams of faecal culture were weighed out, placed onto a piece of double-layer cheese cloth, and closed by a rubber band. This was placed in a funnel (supported by a funnel stand), which was then filled with lukewarm water until the water covered the faecal material. The apparatus was left for 24 hours; then 15ml of fluid were taken from the stem of the funnel into a test tube which was left to stand for 30 minutes. The supernatant was removed with a Pasteur pipette and a drop of the aliquot was transferred to a microscope slide using a Pasteur pipette. A drop of iodine was then added, and the slide covered with a cover slip. Under 100 magnification, the samples were examined and the larvae were identified (Table 1) according to Van Wyk and Mayhew (2013).

Herbage sampling

Herbage samples were cut from different locations in the morning at 6:30-7:00 using scissors. Approximately 300-600g was sampled in a (W) shape in each paddock (Hansen and Perry, 1994). Paddocks were sampled every month and the samples were placed in plastic bags. The samples were transferred into gauze bags and soaked in water for 24 hours. In the first 3-4 hours the grass bags in the water were removed and replaced 5 times then left at room temperature overnight. During the next morning, the bags were removed after running fresh tap water over them into the bucket. The bucket and contents were allowed to stand for an hour. The top of the supernatant was carefully siphoned off, leaving about 1 litre. The sediment was poured into a large funnel stand with the bottom clamp fastened and then left to stand for 1 hour after discarding any heavy debris that sedimented in the first 10 minutes. The sediments (35 ml) were taken and kept in a refrigerator to

cool at 4 ◦C. The third- stage L_3 were identified under a light microscope (100 x) after addition of 1ml of iodine to the cool sediment and 0.2ml of sodium thiosulphate as a counter stain (Hansen and Perry, 1994). The L_3 were counted and identified as per the key of Van Wyk and Mayhew (2013). The dry matter content of the pasture grass samples was determined and results were expressed as the number of L_3 per kg of herbage dry matter (count multiply by 1000/ weight of dry herbage in grams).

Climatic data

An automatic weather station was set by Agrometeorology Department in the experimental site at the Ukulinga research farm which was used to collect standard meteorological weather data, including maximum and minimum air temperature, relative humidity and total rainfall. The data were collected every two minutes then averaged monthly.

Table 1: Differentiation of nematode infective larvae

Nematode species	Total length (µm)	Head	Inside the body	Sheath tail	Other differential features
Haemonchus	650-850	Narrow rounded (bullet-shaped)	16 gut cells	Medium	Tail ending in fine point
Trichostrongylus	560-796	Tapered head	16 gut cells	Short	Smooth larval tail without filament
Strongyloides	650-850	Bullet-shaped	Oesophagus extends to ½ lengthof larval body	Absent	Slender body, larval tail notched
Nematodirus	752-1248	Broad, rounded	8 large intestinal cells	Extremely long	Sheath tail filamentous, larval tail notched or lobed
Cooperia	666-956	Square with re-fractile bodies	16 gut cells	Medium	Tail of larvae rounded with filament

Van Wyk and Mayhew (2013).

Data analysis

The data of the faecal egg counts, larvae counts of the faecal culture, L_3 counts from the grass samples and coccidian oocysts count were analyzed by Repeated Measures Analysis of Variance (MANOVA) using the General a Liner Model (GLM) procedure of SAS (2002) according to the following model:

$$Y_{ijkl} = \mu + M_i + S_j + (M*S)_{ij} + G_k + W_l + e_{ijkl;}$$

Where: Y_{ijkl}= individual monthly observation; µ = overall mean; M_i= monthly effect; S_j= effect of animal species; $(M*S)_{ij}$= interaction between month and animal species; G_k = co-variate effect of initial egg count, W_l = co-variate effect of initial live weight and e_{ijkl}= error of mean.

Results

Climatic conditions

Monthly climatic conditions, including (minimum and maximum air temperature, total rainfall, minimum and maximum relative humidity) during the period of study are presented in Figure 1. The weather conditions during the experimental period showed the highest amount of rain in summer (October to December). There was no rain and the temperature was lowest in winter (May to July).

Nematode faecal egg counts and faecal culture larvae

The effect of seasons on EPG was significant ($P < 0.05$). However, initial live weight and species of animals had no significant effects on the EPG. The EPG showed seasonal variations generally in accordance with rainfall, irrespective of live weight and species of animals during the study period (Figure 2).

In both ewes and does the EPG started to increase in spring (August/ September) to reach the highest level when the rainfall was highest (November/ December). Subsequently, the EPG decreased especially during the dry period (end of May to end of July). No significant differences in the mean EPG were observed between the ewes and the does at any time of the year. The contributions by genus to the count of nematode (L_3) are presented in

Figure 3. Total larvae were greatest during the time of the highest faecal egg counts. *Trichostrongylus spp* (22-24.5%) was the main species revealed in both ewes and does. *Strongyloids* species (19-21%), followed by the most pathogenic species,

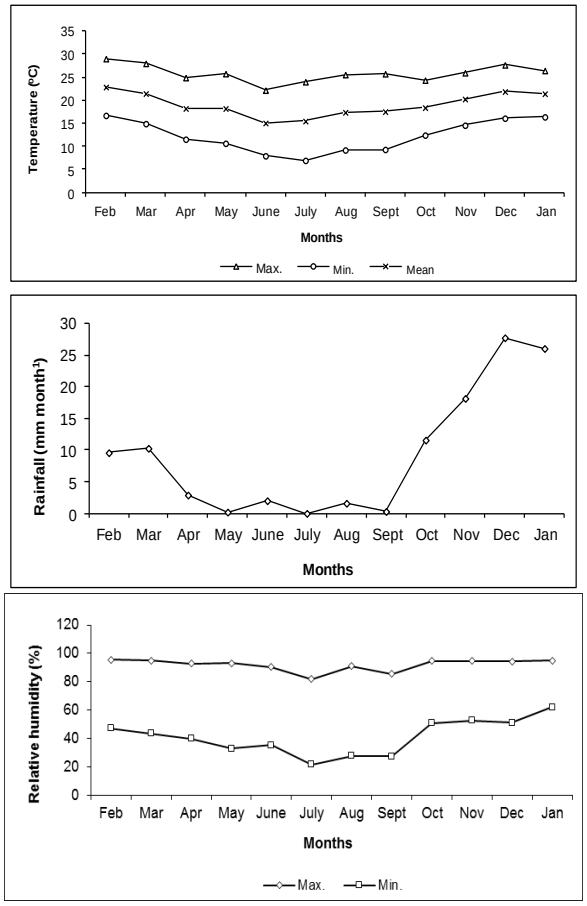

Figure 1: Means and range of temperatures (° C), and relative humidity (%) and mean monthly rainfall (mm)

Haemonchus contortus (14.5-16%) and *Nematodirus* species (13-16%) and finally *Cooperia* species (10-13%). There was a significant ($P<0.05$) seasonal effect on total L_3 from the faecal culture.

Third- stage larvae on pasture

The counts of nematode L_3 larvae recovered from Kikuyu pasture at Ukulinga Research Farm are shown in Figure 4. The overall counts of L_3 on pastures were relatively similar to the pattern of EPG and rainfall. The highest amount of $L_3$was in spring (August), whilst the lowest count was observed in winter (July). The infestation of for does and 956.25 for ewes. In July which had no rain, the mean OPG were 712.5 for does and 537.5 for ewes. The lowest level of OPG was observed in June; 387.5 for does and 412.5 for ewes.

Coccidian oocysts count (OPG)

Group mean OPG varied ($P<0.05$) across seasons (Figure 5). At the beginning of the study OPG mean was 406.25 and 218.75 for the does and the ewes, respectively. In December, which recorded the highest rainfall, the OPG means were 1293.75

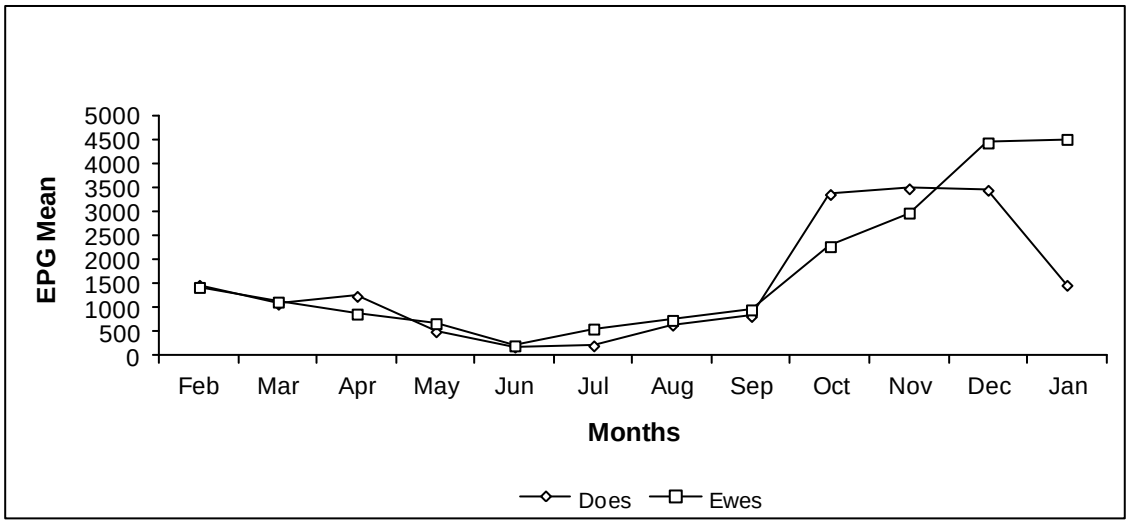

Figure 2: Monthly mean egg per gram of faeces (EPG) for ewes and does during a period of 12 months. SED value was 55. Arrows indicate time of anthelmintic treatments used.

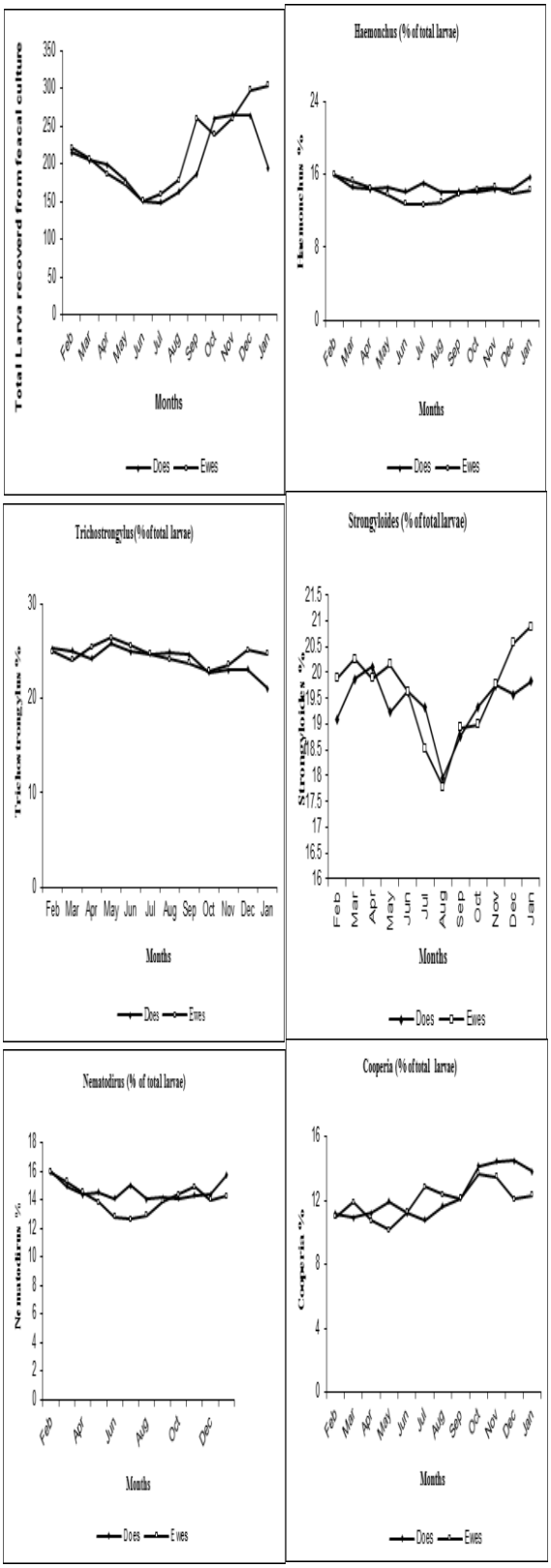

Figure 3: Percentage of nematode infective larvae obtained from the faecal culture. SED values were: Total larvae 0.57, *Haemonchus* species 0.34, *Trichostrongylus* species 0.29, *Strongyloides* species 0.29, *Namatodirus* species 0.28 and *Cooperia* species 0.28.

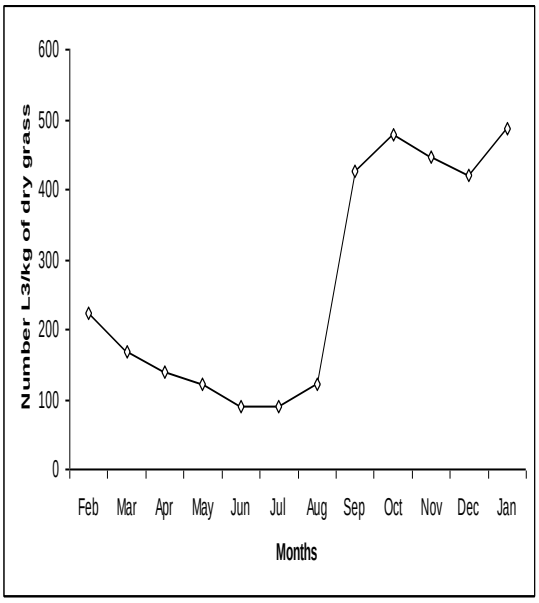

Figure 4: Monthly mean of nematode third stage (L_3) larvae recovered from a Kikuyu pasture during a period of 12 months. SED was 48.4

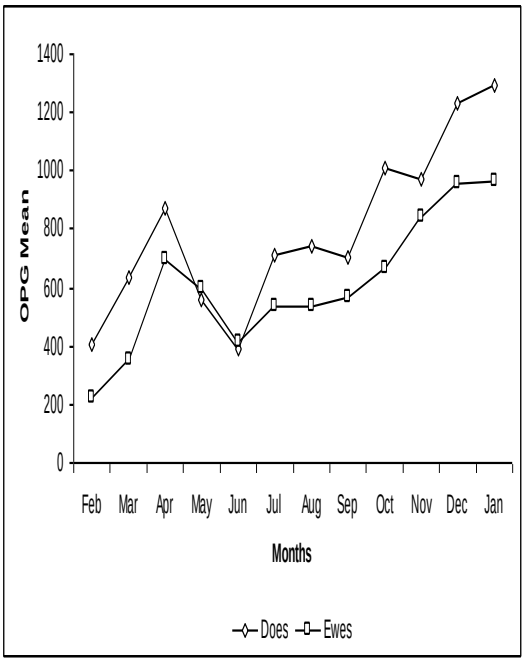

Figure 5: Monthly mean of coccidian oocysts per gram of faeces for ewes and does during a period of 12 months. SED was 112.6

Live-weight variation

The season had no significant effects on the live weight of the experimental animals. The live

weight of ewes and does ranged from 42 to 47kg and 27.5 to 35kg, respectively, during the entire study period. Monthly live weight means are shown in Figure 6.

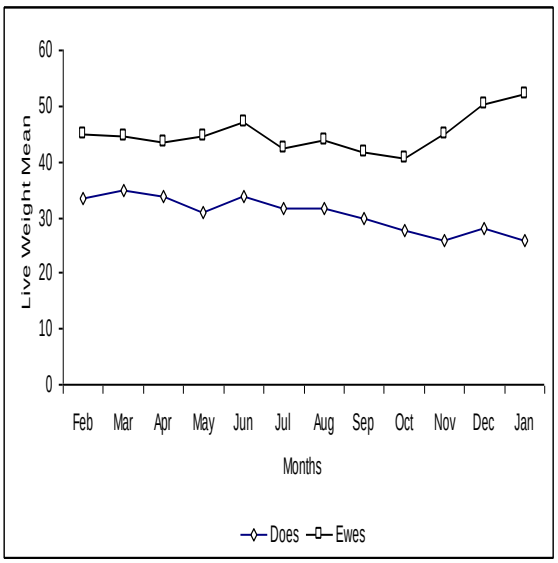

Figure 6: Monthly variation live weight for ewes and does during a period of 12 months (February 2008- January 2009). SED was 2.8 kg

Discussion

The ewes and does observed in this study were infected with a variety of parasites such as nematode parasites and *Eimeria* species. Similar observations have been reported in Ghana (Agyei *et al.*, 2004) and Ethiopia (Regassa *et al.*, 2006). Also the EPG, larval counts from the faecal culture, L_3 from the pasture and coccidian oocysts count were highest in summer (December) and lowest in winter (July), indicating that internal parasite infections had seasonal patterns. These results are in agreement with observations made elsewhere (Tembely *et al.*, 1997; *et al.*, 1998; Vlassoff *et al.*, 2001; Agyei *et al.*, 2004; Waller *et al.*, 2004 and Sissay *et al.*, 2007). The variation in the time to reach peak EPG among the above studies was probably due to the variation in management practice, weather, animal breed or age. December is characterized by rainy, hot and humid ecological conditions that are ideal for nematode infective larvae growth and transitions. Bekele (1991) stated that the weather situation in Sub-Saharan Africa is suitable for the growth of infective nematode larvae.

The prevalence of pasture L_3 in this study was lower in dry months (winter) than in rainy months and is similar to the result reported by Tembely *et al.* (1997). In addition, Stromberg and Averbeck (1999) reported that larval survival varied greatly on pasture; most of the ensheathed infective larvae can survive weeks to months on pastures, depending on the environment and species of nematode. In this study, a quick recovery for the infective larvae on pastures was observed in early spring (August/ September), which agrees with Tembely *et al.* (1996) that eggs will only develop into infective stage when the weather is appropriate.

Results from the faecal culture indicated that *Trichostrongylus*, *Strongyloids* and *Haemonchus* were the most prevalent parasites in both ewes and does throughout the study. Southcott *et al.* (1976) considered *Haemonchus contortus* as a warm climate species. Also Sissay *et al.* (2007) reported that *Trichostrongylus circumcincta* has the ability to survive in adverse conditions within both host and pasture wherever sheep and goats are raised. However, Agyei (1997) reported that the level and number of infective *strongylate* nematode larvae on pasture were directly related to the pattern of rainfall and were also influenced by the number of rain days in the period.

The presence of both *Eimeriaspp* and nematode parasites in this study is similar to that in many studies (Faizal *et al.*, 1999; Agyei *et al.*, 2004 and Regassa *et al.*, 2006). *Eimeria* oocysts count followed the rainfall pattern like the EPG. Similar observations were made in Sri- Lanka by Faizal *et al.* (1999) and in Ghana by Agyei *et al.* (2004). Eggs per gram of faeces in this study had no effect on the live weight gain. This is probably due to the sheep adapting to the infection. The observation is in contrast to Broughan and Wall (2007) where there was negative relationship between live weight and EPG. However, that could be due to the fact that malnutrition and parasitism infections occur concurrently (Koski, and Scott, 2001).

The results have shown the time of anthelmintic treatments used, it clearly gave the idea about the erroneous use of broad-spectrum anthelmintics which are Ivermectin, Closantel and combination of Abamectin and Praziquantel in this particular farm. Dosing frequency is one of the main factors associated with development of resistant strains (Leathwick *et al.*, 2001). The goat group was treated with Albendazole the previous December. Results in January 2009 confirmed lower EPG in the goats faeces compared to the

sheep group. Notable, the use of anthelmintics on this farm is in contrast to the recommended control reported in the study by Tembly *et al.* (1997) who suggested two treatments per annum, firstly treating the adult animals with an effective broad-spectrum anthelmintic at the start of rainy season, with a second treatment recommended at the beginning of dry season. Generally this study showed the parasitological situation at the University of KwaZulu-Natal farm.

Conclusion

The weather of the different seasons had a significant effect on the populations of nematode parasites and *Eimeria* oocyst associated with small ruminants at the Ukulinga Research Farm. Fluctuating counts of nematode parasites did not affect body weight gain. Rainfall and humidity are likely the main factors for the growth of these parasites. However, the data obtained can be used to design an effective control program strategy for internal parasites of small ruminants on this farm. Treatment of all farm animals with an effective anthelmintic in late April should significantly reduce the number of L_3 on pasture when the weather is suitable for the development of eggs into infective larvae. Other treatment should be before and in the middle of the wet season to prevent the rise in faecal egg counts.

Acknowledgment

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