UNIVERSITY OF NAIROBI

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SURVEY OF HELMINTH SPECIES AFFECTING CATTLE AT THE KENYA VETERINARY LABORATORIES VETERINARY FARM, NAIROBI

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A project report submitted in partial fulfillment of the requirements for award of a Degree of Veterinary Medicine from the University of Nairobi

BY

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DECLARATION

I Aondo Wildah K J30/2085/2010 declare that this project is my original work and has not, to the best of my knowledge, been submitted or presented to any other institution for the award of any degree.

SIGNED: ……DATE…….....

I wish to confirm that this project was done under my supervision and the report has my appproval to be submitted for examination as per the University of Nairobi rules and regulations governing examinations.

SIGNED: ……DATE…….....

DEDICATION

I do herby dedicate this research project to my family especially my mother Hellen for her continued advice,financial support, guidance and encouragement. You've always believed in me and taught me to go for the best in life.

To my friends and course mates for your moral and technical support may you also be motivated and encouraged to achieve your dreams and making them realities.

You have moulded me into the person I am today. I will always be indebted to you for all you have done for me. May the Almighty bless you abundantly.

ACKNOWLEDGEMENTS

My sincere thanks is to God Almighty for His sufficient grace in my life.Col 1:17; and He is before all things, and by Him all things consist.

My project supervisor Professor N. Maingi, I thank him for his advice, informed suggestions, guidance and patience throughout the entire period of carrying out this survey.

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ABSTRACT

In the month of March year 2015, I did a survey of helminths species affecting cattle at the Veterinary Farm located at the Kenya Veterinary Laboratories, Kabete.The cattle in the farm are grazed together as one group in open fields except for the calves. I collected fecal samples from the rectum of 20 cattle and proceeded to the laboratory for sample analysis and helminth identification.Using the McMaster floatation technique and microscopy, I identified the eggs present after which I did culture and incubation for seven days for the positive samples only. I then identied the larval stage three that were present. Of the twenty animals sampled, only four were positive for nematode eggs and the egg counts were very low, ranging from 100 to 300 eggs per gram of faeces. Two of the positive animas were male and two were females, three yearlings and one adult. This relatively low prevalence of nematode eggs and low egg counts could be attributed to the dry period during which I collected the samples. The animals could also have been dewormed prior to sampling.There were parasitic helminths present and they were of different species. I was able to isolate larvae of *Haemonchus* and *Oesophagostomum* species from the faecal cultures.

CHAPTER ONE: INTRODUCTION

1.1 Background information

Parasites are organisms that live in (internal or endoparasites) or on (external or ectoparasites) a "host" animal. They can be found on virtually all-living things and in natural conditions generally existing in comparative harmony with their hosts, without breaks of clinical parasitism relatively rare (Brunsdon *et al*., 1975). Domesticated animals harbour a wide range of parasites, the most important of which are internal parasites. The main reason is that the ruminant domestic animals are run in large flocks or herds, concentrated in confined areas that favour the build up of parasite infections. In contrast to the wild state parasite infections in domestic animals especially ruminants result in subclinical or clinical disease symptoms. Helminth parasites are by far the most serious cause of production losses in farmed ruminants (Familton & McAnulty, 1997) and the nematodes are the most important of these.

The epidemiology of the internal parasites is complicated by interactions between the effects of weather on the development, migration and survival of the free-living stages, the variety of mechanisms of host resistance to the parasitic stages, the numerous grazing management practices used by farmers and the number of nematode species involved(Leathwick*et al*., 1992). As many as eight nematode genera may be present in the host at one time (Brunsdon, 1970a; Douch*et al*., 1984).

1.2 Justification

The survey I carried out on helminthes affecting the cattle in the vet farm located at Kabete vet labs has not been documented before. This is a dairy farm whose main aim is to maximize milk

production as well as to improve reproductive indices such as calving rates and intervals. For the past few years, milk production as well as calvings has reduced. Heavy helminth infestation in a herd can be hazardous and lead to the above stated loss and hence the urge to carry out this survey.

1.3 Survey objectives

1.3.1General objective

Identification and documentation of helminths affecting cattle in the vet farm

1.3.2Specific objectives

1.Identification of the helmiths by genus and species.

2. To determine the level of infestation and advice on control.

1.4 Hypothesis

Ho Helminthes are not present in the farm.

H1 Helminthes are present in the farm.

Ho Helminthes present are of the same species.

H1 Helminthes present are of different species.

CHAPTER TWO: LITERATURE REVIEW

2.1 Helminthiasis

Helminths are recognized as a major constraint to livestock production throughout the tropics and elsewhere (Ibrahim *et al*., 1984; Waller, 1987). Among different types of helminthes, nematodes are the most important as far as their prevalence and adverse effects are concerned. They cause retarded growth (Ashraf, 1985; Kochapakdee*et al*., 1995), lowered productivity (Perry and Randolph, 1999), mortality (FAO, 1974; Sykes, 1994) and high economic losses (Irfan, 1984; Iqbal*et al*., 1993).

Most of the parasite control programs are based upon a combination of chemotherapeutic control, grazing management, dietary management, biological control, vaccination and ethnoveterinary treatment (Waller, 1999; FAO, 2002). In the absence of an integrated helminth control program coupled with development of resistance in parasites to several families of drenches (McKenna, 1995; Vermunt*et al*., 1995; Chandrathani*et al*., 1999; Chartier*et al*., 2001; Leathwick*et al*., 2001) results in high prevalence of helminthiasis. Different workers have conducted studies on the prevalence of helminthes in different species of ruminants in different areas of the world. There are many associated risk factors influencing the prevalence and severity of GI helminthes. These include age, sex, weather condition and husbandry or management practices (Khan et al., 2009).

2.2 Factors influencing epidemiology of helminths

2.2.1 Age of the host

The nature, prevalence and intensity of worm infestation may vary with the age of animals. Young animals have often been reported to have higher rates of worm infection and burden

(Asanji& Williams, 1987a; Pal &Qayyum, 1992; Maqsood*et al.,* 1996; Komoin*et al.,* 1999). This may be due to better immune status of the host because of repeated exposure to worm infection in older age (Silverman & Patterson, 1960). A difference in the species involved in the young and old animals has also been reported.

2.2.2 Sex of the host

Most of the researchers have observed higher rates of nematode infection/worm burden in female hosts compared with the males (Asanji& Williams, 1987a; Pal & Qayyum, 1992; Maqsood*et al.,* 1996; Komoin*et al.,* 1999; Valcarcel& Romero, 1999). However, Gulland and Fox (1992) reported that prevalence and intensity of infection (fecal egg counts) were higher in males than females, except during the calving periods, and decreased with age in both sexes.

2.2.3 Climate of the area

The development, survival and transmission of eggs and infective larvae are influenced by climatic and environmental factors such as temperature, humidity and precipitation. The effects of these factors often result in seasonal fluctuation of the availability of infective larvae and subsequently in the prevalence of infections and worm burdens of the hosts. The influence of temperature on the time taken for development of the free living stages was demonstrated by Silverman and Campbell (1959). However, many other factors would also affect development and survival within feces, e.g., consistency, disintegration, and husbandry operation such as harrowing (Reinecke, 1960).

In New South Wales, Waller and Donald (1970) reported that any eggs deposited at a dry time would not develop, as there was too little moisture in fecal matter to prevent desiccation of *Haemonchus* eggs. Likewise, Berbigier*et al.* (1990) found that presence of adequate moisture in

the soil was main factor that influenced the development of free living stages of parasites. The number of strongyloid infective larvae on pasture was high during the period of soon after the rains, and very low or none in the absence of rainfall in the coastal savanna regions of Ghana. The number of infective larvae on pasture was directly related to the pattern of rainfall, but it was also influenced by the number of rain days in the period (Agyei, 1997). It was reported that under hot and dry season *Ostertagia* spp. and *Trichostrongylus* spp. larvae were difficult to develop, but their availability enhanced during the rainy days (Anderson, 1983). Climate, especially temperature and humidity, profoundly influenced the movement of nematode larvae on herbage (Callinan& Westcott, 1986; Krecek*et al.,* 1990). The fecal reservoirs of L3 were the most important means of carryover of infection from the end of one wet season to the beginning of another incubated under optimum conditions of temperature and moisture (Chiejina*et al.,* 1988, 1989). Le Jambre and Whitlock (1973) and Mckenna (1974) reported that low temperatures caused prolonged development of the free-living stages and higher temperatures shortened their development, but it was likely that various geographically distributed phenotypes or strains might have varying responses to temperature changes. For example, *Nematodirus Battus* takes short period in spring for hatching; while *N. filicollis* showed extended period of hatching beginning in autumn, steadily increased in winter and finally attained peak in the late winter (Boag& Thomas, 1975). Likewise, Southcott *et al.* (1976) pointed out that *Haemonchus* and *Trichostrongylus* spp. follow the similar development pattern in summer, while *Ostertagia* spp., in autumn resulted in peak contamination in winter. In addition to many other gross climatic factors, microhabitats and microclimate of free-living nematodes are also responsible for fluctuations in the process of translation of helminthes (Silangwa& Todd, 1964; Thomas, 1974; Armour, 1980). A study in the Eastern highlands Province of Papua (New Guinea) indicated that

nematode infective larvae were plentiful on pasture during both wet and dry seasons (Owen, 1998). The prevalence of different species of nematodes, therefore, has a wide variation due to the climatic differences. In Perak (Peninsular Malaysia),having a wet tropical climate, the monthly populations of *Haemonchus contortus* fluctuated slightly except in May and August during which more worms were found in the tracer animals. The number of *Trichostrongylus* was comparatively high from October to December 1992 and again in March 1993 and low during April and June 1992 (Cheah & Rajamanickam, 1997).

2.2.4 Arrested (hypobiosis) larva development

Larvae may become arrested in development within the host as a manifestation of acquired immunity or may also arrest in development as a result of prior experience of certain adverse environmental conditions. This phenomenon, hypobiosis, has great epidemiological significance. The hypobiotic larvae resume their development and attain sexual maturity when external environmental conditions become favorable. A number of reasons have been suggested for hypobiosis. These reasons may be host resistance (Michael, 1963; Michael *et al.*, 1974, 1975), hormonal changes occurring within the host (Andersen *et al.*, 1965), and/or inherent developmental changes in the infective larval stage, either genetically or environmentally induced changes (Armour*et al.*, 1969 a,b), aging of the infective larvae (Stockdale *et al.*, 1970), and environmental conditions influencing the metabolism of the free-living stages (Blitz & Gibbs, 1972a, b; McKenna, 1973). It was found that marked inhibition of *Haemonchus* at an early fourth larval stage occurred during the winter season in New Zealand. However, a less marked inhibition in *Ostertagia* spp. was indicated while there was no evidence of inhibition in *Trichostrongylus Spp* (McKenna, 1973 The other host factor; such as age and previous experience of infection also play a significant role in causing arrested development (Michael*et*

al., 1979). Altaif and Issa (1983) observed that the proportion of inhibited larvae of *Ostertagia* spp. was markedly high during the dry summer months. It appears that environmental stimulus acting upon pre-parasitic larval stages brought about seasonal inhibition of *Ostertagia*s pp. in Iraq.

2.2.5 Spring rise, peri-parturient rise and/or post-parturient rise in the fecal nematode egg counts

Spring rise or peri-parturient rise and/or post-parturient rise in fecal egg counts of nematodes also have an important epidemiological significance. This is increase in the fecal egg counts of animals around parturition and/or in spring when the environmental conditions for the development of larvae are favorable. The fecal egg counts showed two peaks: the first in March due to the acquisition of larvae during the rainy season and peri-parturient rise, the second in October probably due to maturation of inhibited larvae (Pandey*et al.,* 1990).This phenomenon has been attributed to a variety of reasons. Morgan *et al.* (1951) found that the nematode fecal egg counts were higher if animals are subject to excessive stress such as extremes of weather and poor nutrition. Whereas, Crofton (1958) demonstrated that increased eggs per gram also occurred in Lactating animals and suggested that the increase was associated with parturition and lactation rather than season. The peri-parturient relaxation in immunity (PPRI) to nematode infection is associated with a rise in fecal egg counts during the peri-parturient period (Ette*r et al.,* 1999). The levels of cortisone are known to increase during periods of stress e.g. peri-parturient period. The experimental administration of cortisone to cattle with nematode infections results in an elevated nematode fecal egg count (Armour, 1967). In many parts of the world, parturition of grazing animals is synchronized to occur with the climate favorable to pasture growth and also suitable for development and survival of free living stages of most helminthes (Wedderburn, 1970).

Connan (1972) demonstrated that the host factors were responsible for immunological impairment around parturition and thus resulted in peri-parturient eggs rise. An association, either direct or indirect with circulating levels of the lactogenic hormone "prolactin" was demonstrated by Kelly and Dineen (1973). The maturation of hypobiotic larval forms was proposed to be responsible for post-parturient rise, and *Haemonchus,Trichostrongylus* and *Ostertagia* genera were reported to be the major egg contributors during the spring rise phenomenon (Yazwinski& Featherstone, 1979). The peri-parturient rise in fecal egg counts of worms was also attributed to the breed differences by Courtney *et al.* (1984), who noticed that three exotic breeds showed no peri-parturient rise in fecal egg counts; while domestic breed showed a pronounced peri-parturient rise. The association of lactation with an increased susceptibility to nematode infection resulted in rise in fecal egg counts (Reinecke &Louw, 1989).Rahman and Collins (1992) studied fecal egg counts and serum prolactin concentration in pregnant and non-pregnant animals over a period of 20 weeks. The mean weekly egg counts of the pregnant were significantly higher ($P<0.01$) than those of the non-pregnant. There was a positive linear regression between prolactin levels and fecal egg counts. It was observed by Fleming (1993a, b) that increases in endogenous circulating prolactin during late pregnancy and lactation might contribute to peri-parturient egg rise irrespective of the developmental stage of the parasite.

CHAPTER THREE: MATERIALS AND METHODS

3.1 Area of study

The survey was carried out in a veterinary farm situated within the Kabete Vet Labs which is off waiyaki way .It is 500 meters away from the University of Nairobi's Upper Kabete Campus.

3.2 Study animals

My sample of choice was fecal matter.The samples were collected and transportedto laboratory(University of Nairobi Department of veterinary parasitology,microbiology and pathology) on the same day and later processed. The farm has 78 cattle 8 of which are housed calves.70 are adults consisting dairy cattle and bull calf weaners awaiting to be sold for beef. The sampling involved adults and the yearlings which are put on pasture grazing in open fields.The total sample size(20) for the study was estimated by the formula by Thrusfied (1995):

$N=1.96^2(1-P)/D^2$

Where N=Sample Size

P= Minimum estimated prevalence= 50%

D= Expected outcome 5%

3.2.1 Materials for sample collection and processing

For collection; pd arm-sleeve,water and soap as a lubricant,latex gloves and labels. Samples were collected from the animals' rectum (Figure 1). I employed a floatation technique(McMaster),and later culture and incubation of the positive samples to isolate the larval stage three(infective stage).

Figure 1: The sampled cattle and the investigator collecting faecal samples from the rectum of a cow.

MC MASTER TECHNIQUE.

EQUIPMENTS (Figure 2):

Microscope

Mixing vial (marked with two lines, one at 28ml mark and another one at 30ml mark)

McMaster counting slide

Wooden spatula

Plastic cups

Tea strainer

Syringes

Floatation solution (saturated salt solution)

3.2.2 Procedure

1. Weigh 2gms of feces into a clean labeled fecal cup.

2. Add floatation solution of 28mls into the fecal cup to make a total volume of 30mls.

3. Using a wooden spatula, mix the feces well into solution to make slurry.

4. Pour the solution through a tea strainer into a clean cup. After letting the solution strain for a few minutes, tap the strainer against the cup until you just have a ball of feces left in the cup. Discard feces.

5. Using a dropper or syringe, constantly stir the solution then immediately draw up solution from the TOP of the mixture.

6. Charge one chamber of the slide with the sample in the dropper or syringe by placing the dropper tip at the edge of the slide and discharging sufficient sample between the upper and lower slides to fill the area under the grid. Do it slowly to avoid bubbles forming. Fill the second chamber of the slide with a different drawn sample. Allow the slide to sit long enough to allow the eggs to float to the top, near the gridlines

7. Place the slide on the microscope and observe.

Figure 2: Equipment and faecal samples during the laboratory analysis

3.2.2.1 Examination under microscope

Focus on the grid.

Count eggs inside and under the grid lines.

Record the number of eggs for each grid

Calculating eggs per gram

3.2.2.2 Coproculture

About 10mg of the fecal samples that were positive for helminth eggs were incubated for seven days to isolate the infective larval stage 3.

They were put in a plastic container and few drops of water were added to maintain the moisture. The cap was loosely replaced and the sample placed in an incubator at 27^0c for one week to allow the larvae to hatch.

At the end of this period, some water was added to the sample and allowed to stand for few seconds to stand without mixing and then the water was decanted.

Using a plastic dropper, a drop was put on a slide and a drop of iodine was added to kill the larvae as well as to stain them. A cover slip was put in place and this was then viewed under a microscope.

3.3 Helminth identification

Helminths are identified by use of their differing body structures at both anterior and posterior ends.

Table 1; **Larval identification**

Genus	Intestinal cell number	Head characteristics	Sheath tail characteristics
Nematodirus	8	Broad rounded	Filamentous
Ostertagia	16	Squared	Medium cone
Cooperia	16	Squared	Medium tapering or finely pointed
Haemonchus	16	Squared with refractile bodies	medium offset
Trichostrongylus	16	Narrow rounded	Short cone
Bunostomum	16	Tapered	Short filamentous
Oesophagostomum/chabertia	32	Broad rounded	Filamentous

CHAPTER FOUR: RESULTS

4.1 Egg counts

Table 2 shows the number of cattle from which samples were collected, their identification, age, sex, nematode egg counts and the type of eggs seen. Out of the 20 animas examined, only four were positive for nematode eggs and the counts were low ranging from 100 to 300 eggs per gram of faeces.

Table 2: Egg counts and egg types isolated

Sample no.	Ear tag no	Sex	Age	Results	Egg count	Egg type
1	201	f	adult	negative	-	-
2	4140	f	adult	negative	-	-
3	2764	f	adult	negative	-	-
4	188	f	adult	negative	-	-
5	4029	f	adult	negative	-	-
6	302	m	adult	negative	-	-
7	2753	f	adult	negative	-	-
8	1689	f	adult	negative	-	-
9	306	f	yearling	negative	-	-
10	288	f	adult	negative	-	-
11	313	f	yearling	negative	-	-
12	304	f	yearling	negative	-	-
13	2792	m	yearling	positive	300	strongyle
14	1682	f	adult	negative	-	-
15	317	f	yearling	negative	-	-
16	300	m	yearling	negative	-	-
17	318	f	yearling	positive	200	strongyle
18	k2	f	yearling	negative	-	
19	2736	f	adult	positive	100	strongyle
20	297	m	yearling	positive	100	strongyle

4.2 Larvae identification

Two genera of round worm larvae *Haemonchus spp* and *Oesophagostomum spp.* were identified from the faecal cultures using microscopy Due to the low egg count in Mc Master Technique, the larvae count was low as well. Three larvae were isolated two of which were *Haemonchus* and one *Oesophagostomum.*

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CHAPTER FIVE: DISCUSSION

5.1 DISCUSSION

Of the twenty animals sampled, only four were positive for egg count. Two were male and two were female, three yearlings and one adult. This relatively low egg count could be attributed to the dry period in which I collected the samples or even the animals could have been dewormed prior to sampling.

Fecal egg counts are generally high in the warm wet season when conditions for infection are favorable (Nalubabamba 1996, Fritsche *et al 1993,* Rahman 1992, Schroder 1979). The drop in fecal egg counts in the dry season is thought to coincide with the hypermetabolic state of most worms in the host (Urquhart *et al* 1992, Soulsby 1986). The use of fecal egg counts to estimate the worm burden is questionable (Ndao *et al* 1992, Duwel 1990, Barth *et al* 1991). This is because worms differ in egg laying ability (Wamae and Ihigi 1990). For example *Haemonchus* may produce 5000-15000 eggs per female per day while *Trichostrongylus* will only produce 100-200 eggs per female per day (Hansen and Perry 1994). These results therefore need to be compared with differential larval count, to determine the worms that are involved.

The two genera identified in the study: *Haemonchus* and one *Oesophagostomum* have been documented before in cattle in Kenya (Maingi and Gichigi 1992).

Haemonchus placei (barber's pole worm, large stomach worn, wire worm) *i*nfections show clinical signs of bottle jaw and odema of the ventral abdomen. Adult worms appear reddish in colour due to blood sucking but the uteri and ovaries remain white. Males measure upto 18mm

long, females upto 30mm long. Males have a copulatory bursa with spicules whereas females have a vulva flap with large ovaries. The worms are rather transparent with longitudinal striations. digestive system has a mouth and anus and have a dorsal lancet in the mouth for cutting host tissues. Eggs are ovoid, about 45 by 80 micrometers, have a thin shell and contain 16-32 cells (blastomere.).

Despite the high prevalence,the worm causes little harm in animals older than two years due to the development of immunity(Urquhart *et al* 1992). These older animals therefore continuously contaminate the pastures posing danger to the young and naïve animals.

*Oesophagostomum radiatum (*nodular worm*):* Adults exhibit a cephalic groove at the level of the esophagus as well as a secretory pore (stomum).they have a well developed buccal capsule with a club shaped esophagus. The females range from 6.5-24mm long and males 6-16.6mm. Males have a bell-like copulatory bursa and paired rod like spicules. Eggs are ovoid ranging from 50-100 micrometers in size.

The young animals suffer from the effects of adult worms whereas adult animals are affected by nodules enclosing larval stages. Infection causes anorexia; severe, constant, dark, persistent, fetid diarrhea; weight loss; and death. In older resistant animals, nodules containing the larvae become calcified and caseated decreasing intestinal motility. Intussusceptions or stenosis occasionally occur. The nodules can be palpated per rectum or visualized at necropsy.(Mercks mannual).

These worms have a direct life cycle as shown in Figure 3.

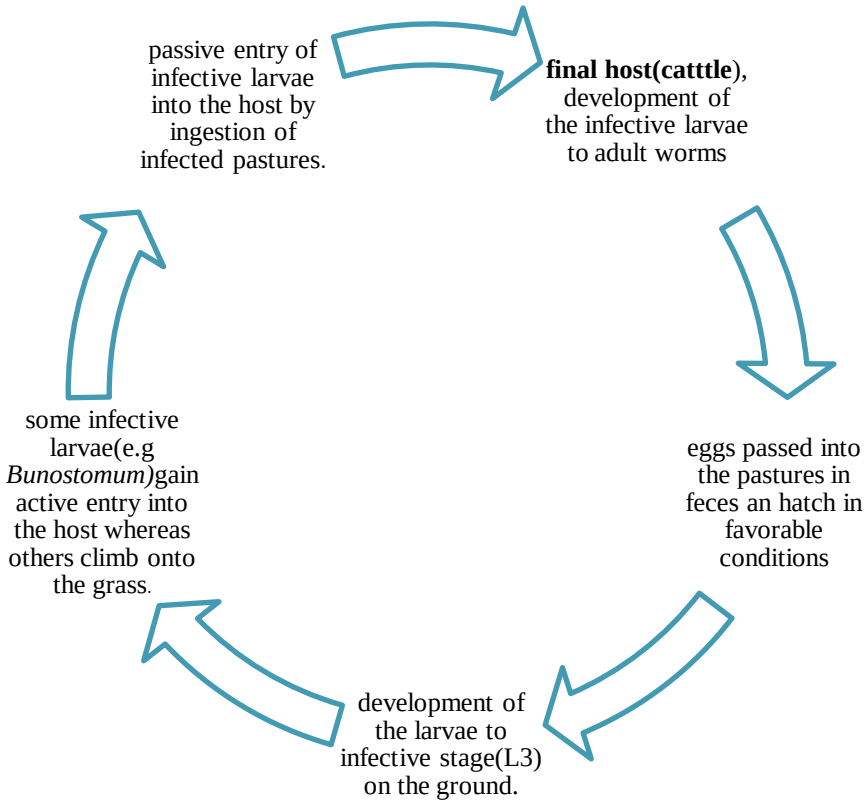

Figure 3 ; Life cycle of gastrointestinal nematodes (Hansen an Perry, 1994; Urquart *et al*,1992; Soulsby 1986; Troncy 1981)

5.2 CONCLUSIONS

The study has shown that there are helminths present in the herd and they are of different species. The egg counts were relatively low and this could be attributed to previous deworming or even more to the dry period within which I carried out the survey. In the dry periods, egg counts are low as compared to the rainy season and these two factors should be put into consideration if a control regime has to succeed. The young are more affected with the helminth infections propably due to their low levels of immunity and naiveness to the parasites.

5.3 RECOMMENDATIONS

1. Based on the above findings, the animals need to be dewormed paying close attention to the younger ones, which are more prone to the risk of infection. Drugs used for deworming should be those to which *Haemonchus* and *Oesophagostomum* are responsive to.
2. Animals should be dewormed just after the rainy season to get rid of infection acquired during the rainy season as well as to help animals withstand the nutritional stress of the dry season. Just before the rains, deworming is done to prevent larval build up in the pastures once the rains start.

   In addition to deworming, the animals should be removed from the contaminated pastures to avoid reinfection. Nutrition should also be improved to enable the animals withstand challenges of reinfection.
3. More survey on the same topic should be done to clearly identify all the possible helminths in the herd and their possible economic importance.

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