CONTROL OF HAEMONCHOSIS USING BARBEVAX® VACCINE IN INDIGENOUS SHEEP AND GOATS IN MELELA WARD, MOROGORO TANZANIA.

NICKSON HASSANALLY NG'UMBI

A THESIS SUBMITTED IN FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF PHILOSOPHY OF THE SOKOINE UNIVERSITY OF AGRICULTURE; MOROGORO, TANZANIA.

2017

ABSTRACT

A cross sectional study was carried out during the dry season (between June and August 2014) to establish the status of helminth infestation in nine traditionally managed small ruminant flocks in Mlandizi village of Melela ward in Mvomero district of the Morgoro region. A longitudinal study was thereafter implemented from August 2014 to January 2015 to determine the influence of *Haemonchus spp.* target Barbevax® vaccine on gastrointestinal parasite burdens in traditional sheep and goats using Barbevax® vaccine. Animals were visited after every 14 days for up to the 19th week. During each visit blood and faecal samples were collected. Vaccination was done four times on days 0, 28, 56 and 98. The vaccine was administered subcutaneously using 1 ml for each animal to be vaccinated. All the study flocks, which were communally grazed and purposively selected, had varying numbers of sheep and goats. The study involved 131 goats and 118 sheep, which were screened for helminth eggs and then the faecal samples, were cultured to recover helminth larvae (L_3) which were identified using morphological features. Out of the animals screened, 54.2% and 67.8% of the goats and sheep examined respectively were positive for helminth infestation. The most prevalent helminth species detected was *Haemonchus* spp. (50.7%). Others were *Trichostrongylus* spp. (29%), *Oesophagostomum* spp. (16.4%), *Cooperia* spp. (5.9%) and *Strongyloides* spp. (3.4%). Results indicated that female sheep and goats had greater packed cell volume (PCV) than males with a significant difference ($P < 0.05$) and with ages 3, 6, 7 and 12 months ($P < 0.05$). At day 56 significant difference was observed between vaccinates and control animals ($P < 0.05$). The observation was that males had their EPGs going down gradually with a significant difference among males ($P < 0.05$). There was a significant difference in EPGs ($P < 0.05$), for days 14 and 42. The mean EPG of males were relatively higher than those of females with a significant difference for both sexes ($p < 0.05$). It was observed further that young

animals (age 2-5 months) had relatively higher EPGs than older animals 6+ months. The mean number of larvae of *Haemonchus* between vaccinates and controls goats was statistically significant ($p < 0.05$) for ages 3 and 9 months. No differences in either total or *Haemonchus* specific egg counts were observed between vaccinates and controls after third vaccination in either sheep or goats. Helminth infestation was shown to be a problem and haemochosis being the most prevalent. The potential of the vaccine efficacy in above associated risk factors is to be further studied in different environments under different infection rates of *H.contortus*. Futhermore, the studies to analyse plasma antibodies should be done to ascertain immune response after vaccination.

DECLARATION

I, **NG'UMBI, NICKSON HASSANALLY**, do hereby declare to the Senate of Sokoine University of Agriculture that this dissertation is my own original work and that it has neither been submitted nor being concurrently submitted for a higher degree award in any other institution.

_____   _____

Ng'umbi Nickson Hassanally   Date

MPhil. Candidate

The declaration is hereby confirmed by:

_____   _____

Prof. A. A. Kassuku. (RIP)   Date

Supervisor

_____   _____

Prof. D. M. Kambarage.   Date

Supervisor

_____   _____

Prof. E. D. Karimuribo   .   Date

Supervisor

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ACKNOWLEDGEMENTS

DEDICATION

This work is dedicated to the Almighty God, source of my strength and wisdom; to my beloved father Hassanally Rashid Ng'umbi, my mother Laurensia Kilasi and my sibling Eva Hassanally Ng'umbi, my sisters; Tumaini, Stella, Rehema and brothers Happyson and Marko Hassanally Ng'umbi.

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LIST OF ACRONYMS AND ABBREVIATIONS

CDC	Centers for Disease Control
CI	Confidence interval
EDTA	Ethylene diamine tetraacetic acid
EPG	Eggs per gram
FEC	Faecal egg count
G	Gram
$L_1...L_5$	Larva stage 1 to 5
Mg	Milligram
Ml	Milliliter
No	Number
OPG	Oocyst per gram
PCV	Packed Cell Volume
Std	Standard
UK	United Kingdom

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background Information

Intestinal parasites are known to be prevalent in sheep and goats in a number of different countries of Africa (Hansen and Perry, 1990). These parasites include nematodes, cestodes, trematodes and protozoa and they are commonly associated with poor production and unthriftiness and can produce acute diseases and even death especially in young animals. The presence of gastrointestinal nematodes in animals, however, does not mean that they are necessarily the cause of overt disease, so it is important to assess the type and level of parasitism in a herd or flock in order to determine the significance of parasite infection and to recommend the most cost effective control measures (Hansen and Perry, 1990).

Infection with gastro-intestinal nematodes remains to be one of the most prevalent parasitic diseases affecting small ruminants all over the world. Parasitic nematodes are responsible for major economical losses in the sheep and goats due to either direct (host mortalities, decreases in production) or indirect (needs for treatments, labour costs, housing) consequences (Sykes, 1994).

Haemonchus contortus is the most important nematode parasite of small ruminants in tropical and subtropical regions, both as a major cause of mortalities due to its cost of control (Besier and Love, 2003). Gastro-intestinal nematodes are almost universally present in any animal examined provided no treatment has been done (Mahlau, 1970). Younger animals tend to be more susceptible to helminth infestation

compared to adults. The most common helminths are in the family trichostrongylidae with *Haemonchus* species dominating in tropical or sub-tropical regions (Dagnachew, 2008) but the prevalence of haemonchosis is higher in goats than in sheep (Dagnachew, 2008).

The need for frequent anthelmintic treatment to prevent death and disease has led to the widespread development of anthelmintic resistance in *H.contortus* in Australia (Besier and Love 2003; Playford *et al.,* 2014) and more widely (Kaplan, 2004), in other parts of the world which has severely limited the effectiveness of control measures. Alternative approaches to control haemonchosis have been sought for many years, one of the methods being vaccination and Barbevax®, a recently commercialized *Haemonchus* vaccine, has proved useful in Australia (Besier *et al.,* 2012).

Coccidia on the other hand are protozoan parasites; most species infecting cattle, sheep and goats. They belong to the genus *Eimeria* and majority of *Eimeria* species parasitize the intestinal epithelium of infected animals. Older animals usually become immune to infection but often remain carriers of coccidia and continue to pass oocysts in the faeces. Young animals become infected by ingesting sporulated oocysts in contaminated food and water. The sporozoites migrate into the intestinal epithelial cells, where they undergo asexual reproduction followed by sexual reproduction resulting in the production of oocysts which pass out in the faeces. Successive infections may cause young animals to excrete large numbers of oocysts that heavily contaminate sleeping and watering places. The multiplication of the parasites in the intestine causes damage to the epithelium whose severity depends on

the number of oocysts ingested. Clinical signs are usually seen only in young animals. A prominent sign of clinical coccidiosis is diarrhoea, which is sometimes bloody. Affected animals have poor growth rates; severely affected animals may die (Hansen and Perry, 1990).

1.2 Problem statement and study justification

There is limited information on the magnitude of parasitic nematode and protozoan infestations in goats and sheep under pastoral extensive production systems in Tanzania. This study was designed to establish the burden and types of gastro-intestinal parasites prevalent in traditional small ruminant flocks in a tropical region of Tanzania. Furthermore due to the resistance of anthelmintics used in Tanzania (Bjorn *et al.,* 1990; Ngomuo *et al.,* 1990; Keyyu *et al.,* 2002), this study investigated on the potential of using the Barvevax® vaccine to control gastro-intestinal parasites under traditional small ruminant management system in Tanzania. Also, even without resistance vaccination is important to prevent clinical disease.

1.3 Objectives

1.3.1 General objective

To determine the influence of *Haemonchus spp.* target vaccine on gastrointestinal parasite burdens in traditional sheep and goats using Barbevax® vaccine.

1.3.2 Specific objectives

The specific objectives of this study were:

1. To determine *Haemonchus spp*. infection rates in indigenous sheep and goats in Melela ward, Morogoro Tanzania

2. To identify factors associated with *Haemonchus spp.* infections in indigenous sheep and goats in tropical areas in Melela ward, Morogoro Tanzania

3. To evaluate the performance of the Barbevax® vaccine in the control of haemonchosis and its influence on other parasite burdens in sheep and goats in Melela ward, Morogoro Tanzania

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Parasitic nematodes and anthelmintic resistance

Parasitic nematodes of the gastrointestinal tract remain a major health constraint of small ruminants under grazing conditions. Until now, control of these parasitic diseases has usually relied mainly on chemical anthelmintics. However, the development of anthelmintic resistances in nematode populations, as well as the public concern on the use of chemicals in flock industry, requires novel and more sustainable methods. These solutions refer to three main principles of action. The elimination of worms from the host remains the main means to control infections. However, methods to more specifically apply treatments targeting the most susceptible animals within flock have been evaluated (Torres-Acosta and Hoste, 2008). Moreover, new advice on how to use anthelmintic has been developed in order to limit the selection of resistant worm populations. Also, the potential interest of non conventional anthelmintics is to be assessed. Secondly, the improvement of the host response against worms rely either on the genetic selection of lines or breeds of hosts or on the manipulation of nutrition to increase host resistance and/or resilience (Torres-Acosta and Hoste, 2008). Lastly, a reduction of host infection by minimizing contact with infective larvae can be achieved through various methods of grazing management (Torres-Acosta and Hoste, 2008).

The emergence of resistant nematodes in a flock does not necessarily mean that all the worm species are resistant to all the available anthelmintic drugs families. However, with time and under certain management systems, the different nematode

species present on one flock can become resistant to all available drugs (including strains resistant to the three main broad spectrum anthelmintic drugs familie) (Van Wyk *et al.,* 1997). Under such circumstances, the attempts to control the gastrointestinal populations with commercial anthelmintic drugs will become unviable either in economical or clinical terms (Van Wyk *et al.,* 1997; Chandrawathani *et al.,* 2004).

In extensive systems, low stocking rates, the incorporation of rangelands, the use of indigenous breeds more adapted to the local environment and the flexibility obtained through the flock management, should allow to rely on rigorous surveillance combined with low input methods. In more intensive systems, it is likely that a minimal reliance on anthelmintics will remain necessary to insure an efficient control (Torres-Acosta and Hoste H*.,* 2008).

2.2 Nematodes of the tropics

In the tropics, the most important nematode species affecting small ruminants are *Haemonchus contortus*, *Trichostrongylus* species, *Nematodirus* species, *Cooperia* species, *Bunostomum* species and *Oesophagostomum* species (Khan, 2005; Smith, 2009). *Haemonchus contortus* commonly known as the Barber's pole worm is a blood sucking nematode parasite, primarily occurring in the abomasum of cattle, (Fentahun and Luke, 2012). *Haemonchus* spp. feeds on blood obtained by damaging the gastrointestinal and the abomasal mucosa. The effects of infection range from inappetence and mild anaemia to mortality, particularly in younger animals (Newton and Munn, 1999).

2.3 Life cycles of Gastrointestinal nematodes

2.3.1 Life cycles of most trichostrongylidae family

The most important and widely prevalent nematodes are the Trichostrongylidae family (*Haemonchus, Ostertagia, Trichostrongylus, Cooperia* and *Nematodirus*), *Bunostomum* and *Oesophagostomum*. The life cycles of most trichostrongylidae, *Oesophagostomum* and *Bunostomum* are more or less similar: the cycles are direct, that is, these nematodes require the definitive host (animal) to complete their life cycles, though *Bunostomum* can penetrate the skin. Adult nematodes inhabit the gastro-intestinal tract of the definitive host. Eggs produced by the fertile female are passed out in the faeces to the ground. Eggs embryonate and hatch into first-stage larvae (L_1) in the soil which in turn moult into second-stage larvae (L_2), shedding their protective cuticle in the process. The L_2 larvae moult into the third-stage larvae (L_3), but retain the cuticle from the previous 2nd stage larva. This double-cuticled L_3 is the infective stage of these nematodes. The time required for the eggs to develop into infective stage depends on temperature, moisture and air (Hansen and Perry, 1990; Molnár *et al.,* 2013).

Under optimal conditions (high humidity and warm temperature), the developmental process requires about 7 to 10 days. In cooler temperatures the process may be prolonged. Ruminants are infected by ingesting the L_3. Most larvae are picked up during grazing and pass to the abomasum, or intestine, ex-sheathing the cuticle of the 2nd stage larva in the process. The L_3 of the trichostrongylidae family penetrate the mucous membrane (in the case of *Haemonchus* and *Trichostrongylus*) or enter the gastric glands (*Ostertagia*). During the next few days the L_3 moult to the fourth stage (L_4) and remain in the mucous membrane (or gastric glands) for about 10 to 14 days.

They then emerge and moult into a young adult stage (L_5). Most Trichostrongylidae worms mature and start egg production in about 3 weeks after infection (Hansen and Perry, 1990; Molnár *et al.,* 2013).

2.3.2 The parasitic part of the life cycle of *Oesophagostomum*

The parasitic part of the life cycle of *Oesophagostomum* requires about 6 weeks to be completed. The infective L_3 penetrate the lamina propria of the intestinal wall and the host response to the infection is an inflammatory reaction which surrounds the L_3 resulting in the formation of fibrous nodules. The larvae emerge into the lumen of the intestine after a period of about 2 weeks and mature in the following 4 weeks. In animals previously infected, the larvae may spend a prolonged period of time (3-5 months) in the nodules. Eventually many of the larvae will die and the nodules may become calcified (Hansen and Perry, 1990; Boomker, 2013; Mehlhorn, 2015).

2.3.3 The L_3 larvae of *Bunostomum* penetrates host's skin

The L_3 larvae of *Bunostomum* infect ruminants when they are ingested or penetrate the host's skin. The larvae are carried in the venous blood through the heart to the lungs, they penetrate the alveoli, are coughed up and then swallowed, and so pass to the small intestine. Larvae moult both in the lungs and when they reach the abomasum or intestine. Eggs are shed into the faeces by infected individuals. The pre-patent period is 7-9 weeks and maximal parasite longevity is 1-2years (Hansen and Perry, 1990).

2.3.4 Egg production.

Adult fertile female nematodes produce eggs. The period between the infection of an animal by ingestion of infective L_3 larvae and the first egg production by the adult female parasite is called the prepatent period. This period is different for different species of parasites, for example for *H. contortus* of sheep and goats is 2 to 3 weeks. For most other gastrointestinal parasites, the prepatent period is about 3-4 weeks. Different species of nematodes have different egg- producing capacities. The individual female *Cooperia*, for example produces many eggs but is not very pathogenic. Females of *Trichostrongylus* are quite pathogenic but produce few eggs. This means that number of nematode eggs in a faecal sample is not an accurate indication of the amount of damage being done by a parasite (Hansen and Perry, 1990; Molnár *et al.,* 2013).

The number of eggs produced by an adult female nematode will also depend on the level of immunity the host possesses (Hansen and Perry, 1990). In addition, adult female nematodes may increase their egg output around parturition, especially in sheep and goats. The number of eggs detected in the faeces also depends on the consistency of the faeces. Diarrhoeic faeces often contain lower number of eggs per gram than formed faeces due to the effect of dilution. In summary, the number of parasite eggs found in the faeces is influenced by; number of adult parasites established in the gastro- intestinal tract, level of host immunity, species of parasite, age of the host, stage of infection, parturition status and consistency of the faeces (Hansen and Perry, 1990; Molnár *et al.,* 2013).

2.3.5 Development and survival of infective larvae in the environment.

The development of larvae in the environment depends upon warm temperature, air and adequate moisture. In most parts of tropical and sub- tropical Africa, temperatures are permanently favourable for larval development in the environment. Exceptions to this are the highland and mountainous regions, such as parts of Ethiopia, Kenya and Kenya, and the winters of South Africa, where temperature many fall below those favourable for the development of *Haemonchus* larvae (Molnár *et al.,* 2013).

The ideal temperature for larval development of many species in the microclimate of the tuft of glass or vegetation is between 22˚ and 26˚C. Some parasite species will continue to develop at temperature as low as 5˚C, but at a much slower rate (Molnár *et al.,* 2013). Development can also occur at higher temperatures, even over 30˚C, but larval mortality is high at these temperatures. The ideal humidity for larval development in this microclimate is 100%; the minimum humidity required for development is about 85% (Hansen and Perry, 1990; Molnár *et al.,* 2013).

The survival of larvae in the environment depends upon adequate moisture and shade. Desiccation due to low humidity and hot environment kills eggs and larvae and is the most rapidly lethal of all climatic factors (Hansen and Perry, 1990). Larvae may be protected from desiccation for a time by the crust of the faecal pat in which they lie or by migrating into the soil (Molnár *et al.,* 2013). The development of infective larvae ingested by an animal during adverse environmental condition may become temporarily arrested in the abomasal mucosa or intestinal mucosa. This

suspension of development helps some nematode parasites survive the dry seasons. Of the three larval stages in the environment (L_1, L_2, and L_3), it is the L_3 which has a protective sheath, that is the most resistant to variations in moisture, temperature and sunlight (Hansen and Perry, 1990; Molnár *et al.,* 2013).

2.3.6 The dissemination of infective larvae.

The parasite's eggs develop into third-stage, infective larvae (L_3) in faecal material. To make themselves accessible to ingestion by ruminants, the larvae have to migrate or be transported from the faeces in which they were deposited on the ground to any nearby herbage. Such movement occurs in two ways: horizontal migration/transport and vertical migration/transport. The horizontal distance L_3 will actively migrate does not usually exceed 5-10cm. Suitable conditions for larval migration occur when rainfall or moisture disintegrates the crust of faecal material and larvae in this material are washed onto herbage. Invertebrates such as beetles many also play a role in the transport of larvae out of faecal pat onto herbage (Hansen and Perry, 1990; Molnár *et al.,* 2013).

Once on the herbage, infective larvae migrate up and down blades of grass assisted by surface tension due to the amount of moisture on the grass. During rainfall and when dew is on the grass, larvae migrate up the herbage. Following evaporation, the larvae migrate to the base of the herbage and even down into the soil. Heavy rain may wash larvae off the herbage and onto the ground. Larvae in water pools may infect animals' drinking water (Hansen and Perry, 1990).

2.3.7 Effect of climate on survival and development of infective larvae.

The development and survival pattern of infective larvae in the environment differs according to the climate. Three broad types of climate are found in tropical and sub-tropical Africa: Humid tropical climate, Savanna type tropical and sub-tropical climate with a long dry season, and arid tropical climate. The humid tropical climate characterizes much of West Africa as well as the regions surrounding Lake Victoria and parts of coastal eastern Africa. This climate provides a more or less permanently favourable environment for the survival and development of pre-parasitic larvae. The savannan-type, tropical and sub-tropical climate with a long dry season is found in much of eastern and, central and southern Africa. As the dry season progresses, the environment for larval development and survival changes from unfavourable to hostile, with populations of surviving preparasitic larvae declining rapidly in open pastures and more slowly in wooded areas where ample shade is available. At the start of the rains, of course, this unfavourable environment is transformed rapidly into a favourable one for the preparasitic larvae. Arid tropical climate characterizes parts of lowland Ethiopia, parts of Somalia, Semi arid central regions of Tanzania and Sudan and much of the Savannah climate, with its sparse vegetation cover, is often permanently unfavourable for preparasitic larval survival. Where vegetation exists, however, short periods of rainfall or irrigation can transform the environment rapidly into a favourable one for the nematode larvae, particularly the highly pathogenic *Haemonchus* (Hansen and Perry, 1990; Rose *at al.,* 2016).

2.3.8 Factor affecting the size of nematode infections.

The size of any gastro-intestinal nematode infection depends on the following five main factors: The number of infective larvae (L_3) ingested by the host, which in turn is influenced by the climate, the amount of protection of larvae provided by vegetation, the livestock density (i.e., pasture contamination level) and the grazing pattern of the ruminants present. Another factor is the rate at which acquired resistance develops in the host, genetic factors, nutrition and physiological stress (e.g. parturition). The third factor is the intrinsic multiplication rates of the parasites present which are controlled by the fecundity, pre-patent period and environmental and survival rates of these species. Management, particularly grazing patterns is another factor and the last is the use of anthelmintics, including the timing and frequency of administration (Hansen and Perry, 1990; Morgan and Van Dijkv, 2012; Verschave *et al.,* 2016).

2.4 Pathogenesis of Nematode Infections

2.4.1 Effect of parasitic larval stages on the host

Considerable damage is caused by fourth-stage larvae (L_4) of abomasal parasites (*Haemonchus, Ostertagia* and *T. axei*) in the mucosa of the abomasum. The L_3 enter the mucous membrane or the glands for about two to three weeks. If large numbers of *Haemonchus, Ostertagia* and *T. axei* larvae enter the abomasum, the host will be affected by: reduced appetite and reduced digestive capability of the abomasum (Hansen and Perry, 1990; Boomker, 2013).

The larvae of *Trichostrongylus* in the small intestine may cause severe damage to intestinal mucous membrane with similar effects. Under certain circumstances,

larvae ingested at the end of a rainy season (in savannah type climate) may remain inhibited in the abomasal glands during the dry season until the next rainy season or until the animal experiences stress, such as that produced when the animal calves/lambs or is sick. The inhibition will then cease, and the L_4 will develop into an adult worm. This development may be accompanied by destruction of the mucous, membrane the extent of which depends on the numbers of inhibited larvae that were present. The L_4 of *Haemonchus* is a blood sucker in the abomasum. Animals infected with large numbers of larvae therefore may suffer from anemia before the parasite eggs can be detected in the animal's faces (Hansen and Perry 1990; Boomker, 2013).

2.4.2 Effect of adult worms on the host

Infections with gastro-intestinal nematodes usually involve several different species of parasites, which may have an additive pathogenic effect on the host. Mixed infections comprising some of these genera *Haemonchus, Ostertagia, Trichostrongylus, Bunostomum, Cooperia, Nematodirus, Oesophagostomum* and *Trichuris* are common. The pathogenic effect of gastro-intestinal parasites may be sub-clinical or clinical. Young animals are most susceptible. The effect of these parasites is strongly dependent on the number of parasites and the nutritional status of the animals they are infecting. The following clinical signs may be seen: Weight loss, reduced carcass quality, reduced wool production/quality, reduced feed intake, diarrhea, blood and protein losses to the gut, anemia, oedema and mortality (Hansen and Perry, 1990; Boomker, 2013).

Severe protein loss into the abomasum and intestine due to damage caused by the parasites often results in oedema in the sub-mandibular region, a condition called 'the bottle jaw'. Some nematode species, especially those that suck blood, such as *Haemonchus, Bunostomum* and *Oesophagostomum,* are responsible for specific clinical signs. *Haemonchus* is the most pathogenic of the blood suckers and infection with large numbers of this parasite often result in severe anaemia in the host. Blood losses from *Bunostomum* and *Oesophagostomum* infections may add to the severity of the anaemia (Hansen and Perry, 1990; Boomker 2013).

It is undeniable that gastrointestinal infections cause economic losses to livestock keepers using grazing and/or browsing feeding systems. Anthelmintic drugs are thus simple, effective way to reduce the losses caused by gastrointestinal infections. However, the flockers' decision to treat animals against gastrointestinal infections is not always based on real evidence related to the role of parasitic infections affecting animal's productivity, health or welfare (Torres-Acosta and Hoste H.*,* 2008).

2.5 Control of Gastro-intestinal parasites

Studies by Cabaret *et al.* (2002), shows that helminth control is based on drugs designed against intestinal nematodes, small and large lungworms, *Moniezia*, *Fasciola* or *Dicrocoelium*. Although anthelmintics have a large array of parasitic targets, none are efficient at normal or even at higher doses on all these parasites. Several benzimidazole drugs are efficient against gastro-intestinal nematodes and small lungworms and *Moniezia* (fenbendazole at 3 times the ordinary dosage of 10mg/kg in small ruminants), nematodes and flukes (albendazole) when used at

higher dosages. Three groups of anthelmintics are available: benzimidazoles (albendazole, cambendazole fenbendazole, flubendazole, mebendazole, oxfendazole) and probenzimidazoles (febentel), imidazothiazoles (levamisole, tetramisole), and macrocyclic lactones (ivermectin). The imidazothiazoles are not as efficient on lungworms and are inefficient on *Moniezia* or flukes: tetramisole at 15 mg/kg of bodyweight in sheep did not reduce the larval excretion much: 60% of faecal larval counts were reduced on day 7 after treatment).

Apart from use of drugs, alternate and rotational grazing can both provide opportunities to reduce dependence on anthelmintic drugs and can easily be complemented with supplementary feeding. More basic knowledge of animal nutrition and gastrointestinal epidemiology under hot humid and subhumid tropical conditions is needed to improve the applicability of nutritional strategies for the control of gastrointestinal infections (Torres-Acosta *et al.,* 2012)

2.6 The helminth vaccines and goal of vaccination.

The most important factor in determining diseases in many helmithiases is the intensity of infection. It follows, therefore, that the goal of vaccination is not necessarily the eradication of infection but a reduction in worm burden sufficient to prevent development of the disease. Unless this concept is clearly and generally understood, the interpretation of the success or otherwise of vaccine trials will not be understood and consumers won't be able to see its value indeed, this was the experience either the commercial vaccine against canine hookworm which was available during the mid 1970's (Miller, 1978). It is unlikely that anti-parasite

vaccines will attain the almost 100% efficacy associated with new anthelmintics and bacterial/viral vaccines (Emery, 1996) but computer modelling of sheep trichostrongylid interactions (Barnes and Dobson, 1990) predicts that adequate control can be achieved with vaccine efficacies of about 80%. Useful levels of protection can be defined as "reducing parasitism below that which causes a significant production loss" (Klei, 1997).

2.6.1 Types of antigens of parasite nematodes

Substantial progress has been made in the last decade in identifying several antigens from *Haemonchus contortus* which, in their native form, stimulate useful levels of protective immunity (70–95% reductions in faecal egg output) in the ovine host. Much work has focused on proteins/protein complexes expressed on the surface of the worm gut which are exposed to the blood meal, and, hence, antibody ingested with it. The antigens generally, but not in all cases, show protease activity and antibody is thought to mediate protective immunity by blocking the activity of enzymes involved in digestion within the worm (Knox *et al.,* 2003).

There are two types of antigens associated with a nematode parasite: (1) soluble excretory and/or secretory (E/S) products; and (2) those fixed at external surfaces or within the parasite (the so-called somatic antigens). Some of the E/S products and exposed somatic antigens induce an immune response in the host during the course of infection and are designated natural antigens, while antigens that do not induce an immune response during infection are designated hidden antigens as they are hidden from the afferent immune system (Munn, 1997). To be of value as a vaccine, parasite target antigens have to be accessible to antibodies and possibly other immune

response components induced by vaccination. Antigens associated with the parasite gut surface, even though they are exposed to host blood in the case of blood-feeding parasites, are hidden antigens because they are not presented to the immune system during infection. The particular value of hidden antigens in vaccines is that there has been no selection pressure for evolution of parasites with mechanisms for evading the immune response to these antigens (Newton and Munn, 1999).

2.6.2 The hidden antigen approach to vaccination

A hidden antigen is defined as one which is not recognised by the host following infection. Hosts are usually not exposed to proteins on the gut membrane of non-invasive metazoan parasites such as ticks and gastrointestinal nematodes. If, however, these species are also blood feeders, the surface of the parasite intestine is exposed to host immunoglobulin and potentially to antibodies directed against it (Knox *et al.,* 2003). Immunological targeting of gut membrane proteins was pioneered in ticks and this work led to the launch of a commercial vaccine against *Boophilus microplus*, the cattle tick in Australia (Willadsen *et al.,* 1995).

The same approach has shown considerable promise for blood feeding *Haemonchus contortus* in a series of trials conducted with different antigens over the last decade (Smith *et al.,* 1999). The late larval and the adult stages of *H. contortus* ingest host blood and an artificially induced systemic antibody response directed to certain components expressed on the surface of the worm gut is clearly detrimental to the parasite. The degree of vaccine immunity is highly correlated with antibody titre (Munn *et al.,* 1997; Smith *et al.,* 1999) and can be passively transferred to naive

sheep by serum (Smith, 1993) or to lambs by colostrum (Andrews *et al.,* 1995) indicating that it is antibody mediated. The most effective gut antigens have been identified as enzymes presumed to be involved in digestion of the blood meal. Since some of these enzymes can be inhibited by vaccine antibodies in vitro (Smith *et al.,* 1997), the same mechanism is thought to operate in vivo, although it is also possible that the accumulation of antigen–antibody complexes on the intestinal surface may act as a barrier to nutrient absorption (Newton and Munn, 1999). Either mechanism would lead to worm starvation, resulting in reduced egg output, weakness and ultimately an inability to avoid being flushed through the pylorus during a peristaltic contraction.

Not surprisingly, the mechanisms and manifestations of immunity induced by hidden antigens are completely different from that which is acquired naturally. For example, sheep which have experienced several weeks of continuous exposure to *Haemonchus*, are able to prevent most incoming larvae from establishing, whilst the majority of those which do manage to establish become arrested as early fourth stage larvae (Barger *et al.,* 1985). By contrast, larval establishment is unaffected in sheep immunised with *Haemonchus* gut membrane proteins (Smith and Smith, 1993), rather egg output is severely reduced, coupled with a loss of adult worms, especially females (Smith *et al.,* 1994). Selective loss of female worms is not observed in sheep naturally immune to *Haemonchus*. Hence, hidden antigens are thought to be less likely to be subjected to selection pressure, reducing the risk of antigenic variation, but repeated vaccination will be required as specific host immune responses would not be boosted by subsequent natural infection. However, hidden antigen protection

can persist for several weeks (Andrews *et al.,* 1997) until sheep are capable of regulating *Haemonchus* through natural immunity (Barger *et al.,* 1985) and there is experimental evidence that hidden antigen immunity does not interfere with naturally acquired immunity (Smith and Smith, 1993). Other economically important genera such as *Ostertagia* and *Teladorsagia*, though not obligate blood feeders, do contain host immunoglobulin (Murray and Smith, 1994), presumably obtained by ingesting plasma proteins resulting from the host inflammatory response to the parasite. Significant protective effects have been reported in cattle immunised with gut membrane proteins from *Ostertagia ostertagi* (Smith *et al.,* 2000), although little protection was observed when the same experiment was done with *Teladorsagia* in sheep (Smith *et al.,* 2001).

2.6.3 Barbervax [®] vaccine profile

Barbervax [®], the first vaccine in the world for a worm parasite of sheep and a revolutionary new tool for flockers to combat Barbers Pole was registered for use in Australia in early October 2014. The first batch of vaccine, consisting 300,000 doses, was all sold within 10 days just by word of mouth. The basis for Barbevax[®] was devised after many years of research at Moredun Research Institute and commercialised during the last five years through a collaboration with the Albany laboratory of the Department of Agriculture and Food, Western Australia, where it is made by Wormvax Australia, a subsidiary of Moredun. Barbevax[®] was trialled extensively in Australian Merinos with good results. Mathematical modelling indicates that the degree by which the vaccine reduces worm egg output and hence pasture contamination offers a level of control superior to a

conventional anthelmintic programme (http://www.moredun.org.uk/news/moredun-launches-barbervax-vaccine-barbers-pole-worm).

2.6.4 Packaging and administration

Barbevax® is sold in 250ml packs like those used for Clostridial vaccines. The dose per sheep is 1ml injected under the skin, irrespective of body weight. It can be given at the same time as other vaccines (though at a different injection site), drenches and remedies (http://www.moredun.org.uk/news/moredun-launches-barbervax-vaccine-barbers-pole worm).

2.6.5 Safety of Barbevax® vaccine

Barbevax® is safe for young lambs and heavily pregnant ewes alike. It should be stored refrigerated but not frozen and has a shelf life of at least 2 years. Barbevax® works against all Barbers Pole worms including drench resistant ones. It offers a more sustainable form of control, because it is extremely unlikely that vaccine resistant worms will develop. Using Barbevax® will reduce the need to use those drenches which are still effective on a particular flock, therefore prolong their life (http://www.moredun.org.uk/news/moredun-launches-barbervax-vaccine-barbers-pole-worm).

2.6.6 Mode of action of Barbevax® vaccine

Barbevax® contains tiny amounts of protein purified from the lining of *H. contortus* intestines. Like all vaccines, it works by stimulating the natural immune response in the animal after injection. The antibodies produced circulate in the animal's blood,

so that the parasites ingest antibodies within blood meal. These antibodies attach to the lining of the *H. contortus* wom intestine, blocking digestion and starving the worm so that it produces far fewer eggs and dies (http://www. moredun.org.uk/news/moredun-launches-barbervax-vaccine-barbers-pole-worm)

2.7 Identification of gastro-intestinal parasites of ruminants.

One of the difficulties in identifying gastro-intestinal parasites of ruminants is that nearly most of the intestinal nematodes look alike. They can, however, be identified down to a species level by microscopical examination. In addition, most of them live in specific sites in the intestinal tract, which helps in the identification process and also their morphological characteristics and sizes of adult worms. Since different species have different pathogenic effects, it is important to know which broad groups are present in a herd or area. Furthermore, some of these parasites have very different development pattern, both outside and inside host, a knowledge which may be important for planning effective control measures (Hansen and Perry, 1990).

Studies by van Wyk and Mayhew*,* (2013) show that the only practical method available to the helminthologist for obtaining an indication ante-mortem of the worm genera with which cattle and small ruminants are infected is to identify the larvae that are found in fresh faeces or that develop in cultures of the faeces of the animals. However, even though the infective larvae (L$_3$) of the common worm genera are generally more easily identified than the ova, even this is often feasible only for the experienced person; distinguishing features such as the shape of the "head" (cranial extremity) of the larva or the length of the sheath "tail" (the extension of the sheath

from the tip of the larval caudal extremity to the tip of the tail of the sheath, are similar to all but the practiced eye. While the first-stage larvae (L_1) of protostrongylids or the third-stage larvae of strongyles (L_3) can be measured, it is not practical to measure each larva when doing routine differential diagnostic counts.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area:

The study was carried out in a purposively selected village of Mlandizi located in Melela ward of Mvomero district in Morogoro region. The village was selected as it has a good number of traditional small ruminants kept by the Maasai who normally don't deworm routinely and rarely seek veterinary help on disease managements. Mvomero is one of the six districts of the Morogoro region of Tanzania. It is bordered to the north by the Tanga region, to the northeast by the Pwani Region, to the east and southeast by Morogoro Rural District and Morogoro Urban District and to the west by Kilosa District (National Bureau of Statistics, 2012). The Maasai of this village traditionally keep cattle, sheep and goats. They also keep dogs and cats with a number of chickens for some. They graze their small ruminants communally with each flock having its own earthen 'boma', a traditional animal and/or human house. During dry seasons, they normally migrate to a distant grazing area with reasonable pastures and return during rainy season. These Maasai are agropastoralist though they base mostly in animal keeping. They are living in close contact with other tribes who are either agropastoralists like the Kamba and Sukuma or crop producers like Waluguru.

3.2 Study design and sampling

Both cross-sectional and longitudinal study designs were used. A cross-sectional study design was employed to quantify the magnitude of helminth infestation in goats and sheep. Three-stage sampling approach was adopted. Stage one involved

purposive selection of households (flocks) with high populations of goats and sheep in Mlandizi village. Stage two involved simple random selection of households with sheep and goats having positive results of eggs per gram (EPGs) and the last stage involved selection of systemic allocated vaccinate and control sheep and goats for a period of 140 days longitudinally. Animals were sampled after every 14 days for 11 times (Day 0 to Day 140). Blood and fecal samples were collected during each visit. Vaccination was done four times on days 0, 28, 56 and 98. The vaccine was administered subcutaneously, 1 ml for each animal to be vaccinated.

3.3 Sampling and Sample size.

The study recruited seven flocks which had animals with EPG ≥ 300 and which the owners agreed to participate in the study. The sample size was calculated using the formula by Thrusfied (2005) $n = \underline{Z^2 \times p\,(1\text{-}p)}/\,d^2$. Where; n = required sample size, Z = confidence level at 95% (std value of 1.96), p = estimated prevalence (50% for unknown prevalence) and d = margin of error (5%). The prevalence of helminth infestation (strongyle) was 30.3% in Ethiopia (Dagnachew, 2008) and that coccidiosis in Tanzania is 40-90 % (Kusiluka *et al.,* 1996) respectively. Estimated prevalence of 30.3% was adopted giving a total of 232 animals to be sampled. However a total of 249 small ruminants were screened comprising of 131 goats and 118 sheep. The distribution of animals sampled is summarized in Table 1.

Table 1: **Distribution of animals sampled in each flock/flock**

Flock number	Sheep			Goats		
	Male	Female	Total	Male	Female	Total
1	2	13	15	10	25	35
2	4	16	20	6	4	10
3	0	0	0	0	5	5
4	3	11	14	4	6	10
5	1	12	13	5	5	10
6	4	11	15	1	5	6
7	13	11	24	9	15	24
Grand total	**27**	**74**	**101**	**35**	**65**	**100**

3.4 Selection of animals for the trial

A random number generator (ProMESA v1.62, Massey University, Epicentre software for sample size calculation) was used to allocate all animals to the two groups. A total of 89 animals (Goats = 43, sheep = 46) with EPG $\geq$ 300 were used in the *Heamonchus* vaccination trial. Vaccinated goats and sheep were 20 and 25, while 23 and 21 were controls respectively. The distribution of animals under trial is presented in Table 2.

Table 2: **Distribution of sampled animals in flocks and those used for the vaccine trial**

Flock No.	Total Animals	Total no of animals Sampled		Total no of animals vaccinated	
		Goats	**Sheep**	**Goats**	**Sheep**
1	50	35	15	14	5
2	30	10	20	7	7
3	5	5	0	2	0
4	24	10	14	7	11
5	23	10	13	2	5
6	21	6	15	3	10
7	48	24	24	8	8
Grand **Total**	201	**100**	**101**	**43**	**46**

Table 3: **Distribution of vaccinate and control animals used in the trial**

Flock No.	Goats		Sheep		
	Vaccinated	Control	Vaccinated	Control	Total
1	10	4	5	0	19
2	2	5	3	4	14
3	0	2	0	0	2
4	4	3	5	6	18
5	0	2	3	2	7
6	2	1	5	5	13
7	2	6	4	4	16
Total	**20**	**23**	**25**	**21**	**89**

3.4.1 Copro-culture groups

The faecal samples were pooled for sheep and goats to establish the copro-culture groups. A total of four groups stratified by species and vaccination goup (namely control and vaccinated animals) were adopted and members in each group were allocated to their respective group using a systematic random approach. More information on the groups is described in Appendix 1.

3.5 Data collection

3.5.1 Collection, storage and transportation of faecal samples

The study animals sampled were initially identified by using ear tags. During sampling, biodata of each animal including approximate age, sex and colour was recorded. Faecal samples were collected *per rectum* using a gloved hand which was subsequently labelled to identify each animal. Faecal samples were stored in a cool box with ice packs and shipped to the helminthology laboratory at the College of Veterinary Medicine and Biomedical sciences of Sokoine University of Agriculture (SUA) for further processing.

3.5.2 Faecal processing for determination of EPG and OPG

The number of eggs per gram (EPG) of faeces for each goat or sheep was determined by McMaster technique as previously described by Hansen and Perry (1990). In brief, four grams of faeces was weighed and placed into a container into which 56 ml of the flotation fluid (saturated Sodium Chloride solution) was added. The contents were mixed well by thorough stirring. The faecal suspension was then filtered through a tea strainer or double-layer cheese cloth before transferring the filtrate into

the second container. Both sides of the McMaster counting chamber were loaded with the filtrate using Pasteur pipettes. The counting chambers were allowed to stand for five minutes before being examined under a microscope at 10X magnification. All the eggs within the engraved area of both chambers were counted. The number of eggs per gram of faeces (EPG) was calculated using standard procedure (Hansen and Perry, 1990). Pooled faecal samples in flocks were cultured for larvae identification at each sampling.

3.5.3 Culturing of faecal samples for identification of helminths

Ten grams (10g) of faecal sample was taken into a mortar. Few drops of water were added to the faeces followed by breaking up the feaces finely using a stirring device. Vermiculate was added to each sample to aid air pores. A plastic cup was cut half way such that the upper half part was removed to remain with the bottom half and below it, at the bottom, small holes were pierced and the faecal sample was put without pressing. Water was filled into a plastic cup filling it at about one-eighth of its volume height. The resized plastic cup was covered with the cloth gauze and stoppered it inversely with the remaining half piece of the plastic cup. The sample above was placed into the full plastic cup which had water. The sample culture was left at room temperature for 7 days during which water was checked regularly.

3.5.4 Recovering of the larvae (Baermman's technique)

Baermann's technique was used for larvae recovery as described previously by Hansen and Perry (1990). Briefly, water from each full cup cultured sample was taken into a conical flask. The remaining was mixed with tap water and added to a conical flask. Each faecal sample was then suspended on top of each conical flask

where by running tap water was filled in each faecal sample to make the entire faecal sample to be immersed into the water. The set up was left overnight to allow larvae to settle down. Then, a sealed pipette at top by a thumb was introduced in each flask and unsealed to collect larvae at the bottom. The contents were then released into a tube container, where a drop of it was taken by a pipette onto a clean microscope slide. A drop of Lugol's iodine was added to kill the larvae and then covered by a microscope cover slide. Examination was done using a compound microscope at 10x magnification. Larvae were identified according to Table 3.4 Key to infective nematode larvae of sheep and goats (Adapted from Dikmans and Andrews (1933), (Hansen and Perry, 1990).

3.6 Blood sampling

Whole blood was collected for packed cell volume (PCV) and plasma extraction (at least 0.5ml). Blood samples were collected from the jugular vein using EDTA vacutanier tubes. Throughout the study period, animals were visited after every 14 days for 11 times (i.e. between Day 0 and Day 140). Livestock keepers were requested to report on any abnormal signs in the flocks so that at any given time it was possible to visit and examine sick animal(s) or any other problem(s) that was reported during the study.

3.6.1 PCV determination

Packed cell volume (PCV) was determined using the haematocrit centrifuge technique (Hansen and Perry, 1990). There were two groups in each of sheep and goats; vaccinate and control groups.

3.6.2 Management of animals

The study animals were communally grazed and allowed for exposure to natural infection by L_3 of different worms in the pasture. To avoid effects of ectoparasites in the animals, before Day 0, acaricides were used to control ectoparasites so that anaemia resulting from them should not affect PCV which may be due to helminthes. Any sick animal was given proper care and attention.

3.7 Administration of the vaccine

The vaccine was administered subcutaneously, 1 ml for each animal to be vaccinated. It was done four times out of 11 times sampling days, from day 0 to day 140. Sampling was done after each 14 days.

3.7.1 Vaccination schedule

The sampling and vaccination was done as shown in Table 4. Fecal samples were used to establish fecal egg count (FEC) while blood samples were used to harvest plasma and also packed cell volume (PCV) of each animal was recorded.

Proportion =   Number of goats/ or sheep testing positive.

Total number of tested goats /or sheep.

Statistical difference between proportions in different groups was compared by Chi-squared test at 5% critical probability. Tables and figures were made using Microsoft® softwares; Word and Excel on Wondows 8.

CHAPTER FOUR

4.0 RESULTS

4.1 Proportion of animals infested with helminths and coccidia

Results for eggs shed by nematodes and cestodes are shown in Table 5. Infestation of sheep and goats with protozoa parasites is presented in Table 6. Nematode eggs were most common, *Haemonchus* being the most prevalent species after faecal culture. The proportion of helminths and coccidial infections in goats and sheep stratified by sex and age of animal is summarized in Tables 5 and 6, respectively.

Table 5: Proportion of goats and sheep with helminth infestations (strongyle eggs) at Melela-Mlandizi village;

Variable	Level	No. positive	Proportion (%)	95% CI	Chi-Square	P-value
Species	1. Goats(n=131)	71	54.2	45.27-62.93	4.79	0.0286
	2. Sheep(n=118)	80	67.8	58.57-76.10		
Sex	Goats 1. Male(n=38)	23	60.5	43.39-75.96	0.73	0.039
	2. Female(n=93)	47	50.5	39.97-61.07		
	Sheep 1. Male(n=33)	15	45.5	28.11-63.65	4.62	0.0315
	2.Female(n=85)	57	67.1	56.02-76.87		
Age category (months)	1. Goats≤6 months (n=54)	28	52.0	37.84-65.66		
	2. Goats>6 months (n=77)	34	41.2	32.84-55.93	0.74	0.3869
	3. Sheep≤6 months (n=60)	43	72.0	58.56-82.55		
	4. Sheep>6 months (n=59)	33	60.0	42.40-68.85	3.16	0.0752

Table 6: Proportion of goats and sheep with *Eimeria* oocysts coccidial infestation at Mlandizi village;

Variable	Level	No. positive	Proportion (%)	95% CI	Chi-Square	P-value
Species	1. Goats(n=131)	69	52.7	43.77-61.46	10.57	0.0011
	2. Sheep(n=118)	38	32.2	23.90-41.43		
Sex	Goats 1. Male(n=38)	31	81.6	65.67-92.26	11.39	0.0007
	2. Female(n=93)	46	49.5	38.93-60.03		
	Sheep 1. Male(n=33)	20	60.6	42.14-77.09	4.03	0.0446
	2.Female(n=85)	34	40.0	29.52-51.20		
Age category (months)	1. Goats≤6 months (n=54)	29	53.7	39.61-67.38	0.41	0.5258
	2. Goats>6 months (n=77)	40	52.0	40.26-63.48		
	1. Sheep≤6 months (n=60)	38	63.0	49.90-75.41	22.38	0.00002
	2. Sheep>6 months (n=59)	12	20.3	10.98-32.83		

Helminth infestation was significantly higher in sheep (67.8%) than in goats (52.7%), while coccidial infections were less prevalent in sheep (32.2%) than goats (52.7%) and these differences were statistically significant. Male goats (81.6%) were more infected with coccidia oocysts than female goats (49.5%), and the difference was statistically significant ($p<0.001$). Similarly, male sheep (60.6%) were significantly more infested with protozoa than female sheep (40.0%) ($p<0.05$). Within both host species the proportion of young animals (less than 6 months of age) containing worms or coccidians was higher though the difference was not statistically significant, except in the case sheep with coccidial infestation.

4.2 Types of *Eimeria* species prevalent at Mlandizi village

The pooled faecal culture for goats and sheep in all flocks screened revealed that the most prevalent *Eimeria species* was *E. caprina* (20.6%), the percentage of other species of *Eimeria* that occurred is shown in the Figure 1.
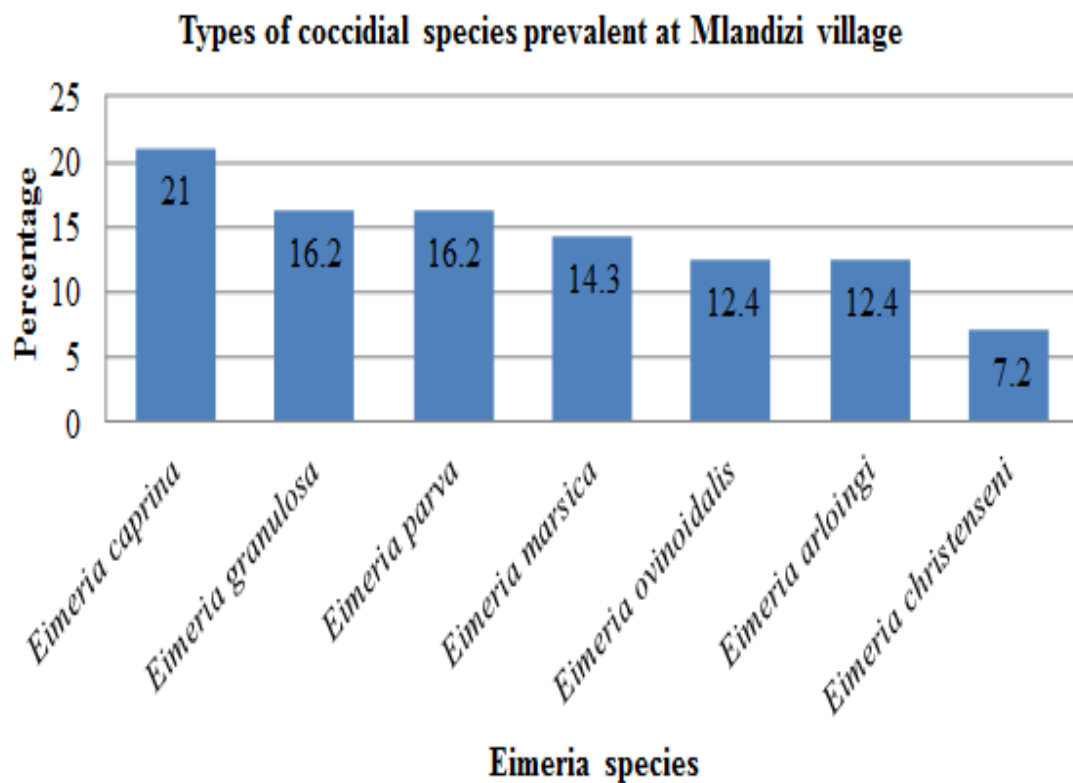
Figure 1: Eimeria species prevalent at Mlandizi village (n = 105)

4.3 Moniezia species identified

Moniezia spp. segments were identified in some of the faecal samples of both sheep and goats and their proportion was 5.8% and 0.8% in sheep and goats, respectively.

4.4 Types of third stage larvae (L₃) recovered from faecal culture

Third stage larvae (L₃) recovered from each pooled treatment group faecal cultures were identified to genus level. *Haemonchus spp*. was the most prevalent (50.7%) of all identified larvae. The results of identified larvae are summarized in Figure 2.

Proportion of Larvae stage 3 identified in Mlandizi

Genus	Percentage
Strongyloides spp.	3.1
Cooperia spp.	4.2
Oesophagostomum spp.	15.2
Trichostrongylus spp.	26.8
Haemonchus spp.	50.7
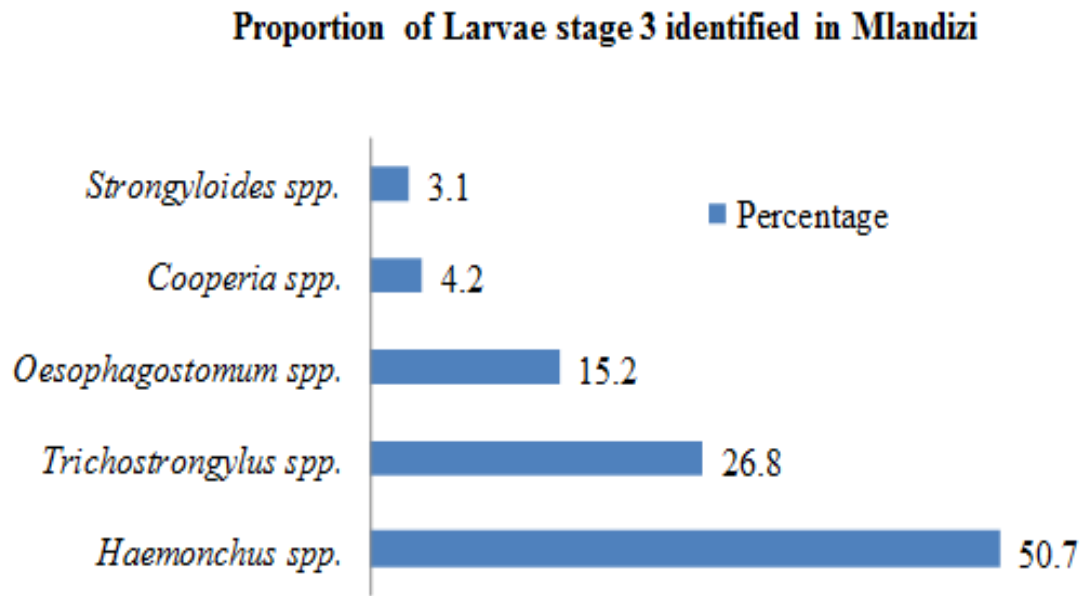
Figure 2: Proportions of larvae stage 3 (L₃) identified in Mlandizi village (n=693)

4.5 Barbevax® efficacy summary

The lambs and kids in this trial belonged to several owners, but all grazed in the same grazing lands, even though they were split into each owner's small flock. It was therefore assumed that all were exposed to the same degree of parasite challenge and so the egg count data from the four groups (sheep or goats; vaccinated or not) were pooled irrespective of owner. This method gave larger group sizes (n=19 to 25). Similarly, the percent of *Haemonchus* eggs at each sampling time was

calculated from larval culture data pooled for each treatment group. The trend of EPG counts in vaccinates and control animal's is presented in figure 3. The EPGs decreased in both vaccinated and control animal after first vaccination and decreased further after the second one before increasing and fluctuating afterwards, finally decreasing at the end. No differences in either total or *Haemonchus* specific egg counts were observed between vaccinates and controls after third vaccination in either sheep or goats.
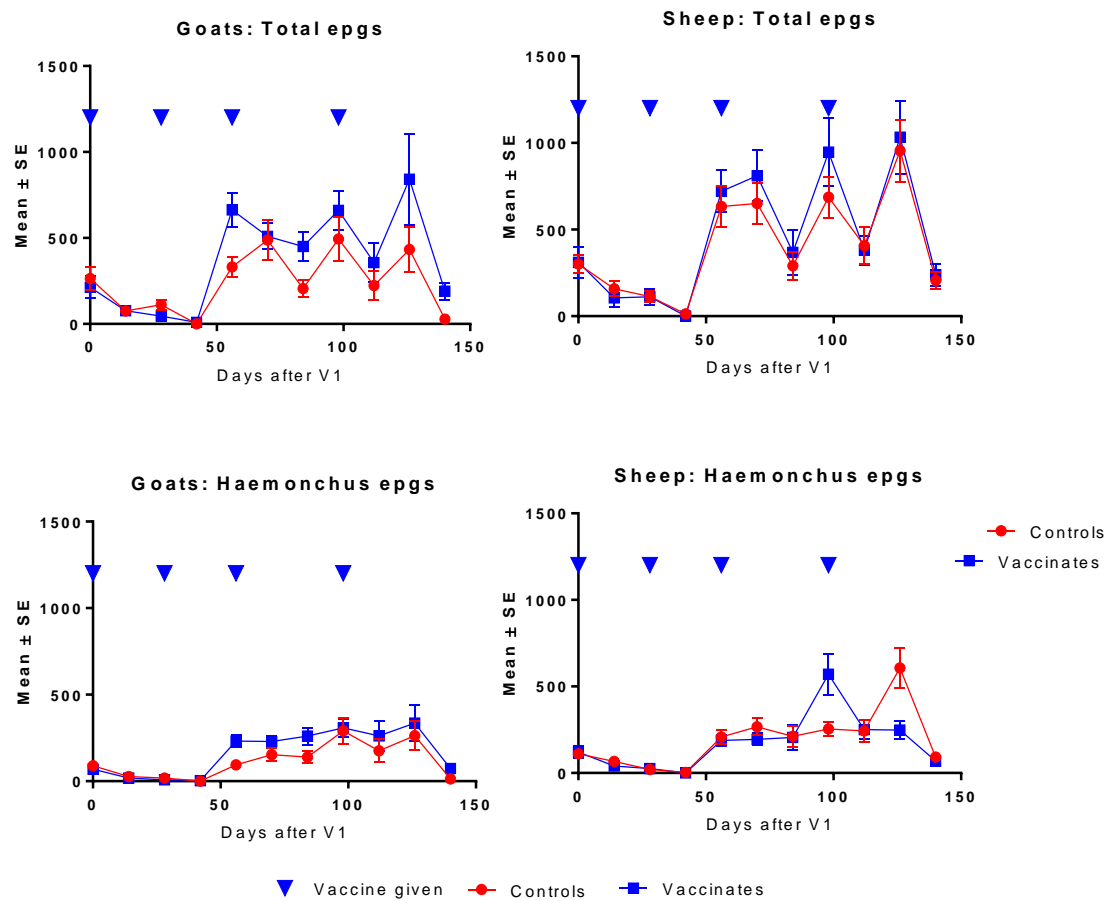
Figure 3: Trends of EPG in vaccinated and control animals s during the study.

4.5.1 PCV trends during the trial period

During the trial period, PCVs for all animals irrespective of vaccination status were above 25%. With exception of days 70 and 112, all other days had the general trend of animals of ages 1 to 3 months with highest PCVs compared with other age groups. At day 56 a significant difference was observed between vaccinates and control animals (P< 0.05).Vaccinated animals had higher PCVs than control animals for the age group of 1 to 3 months, no significance was observed for the rest of the groups. The summary of other days and general trend is shown in the Figure 4.
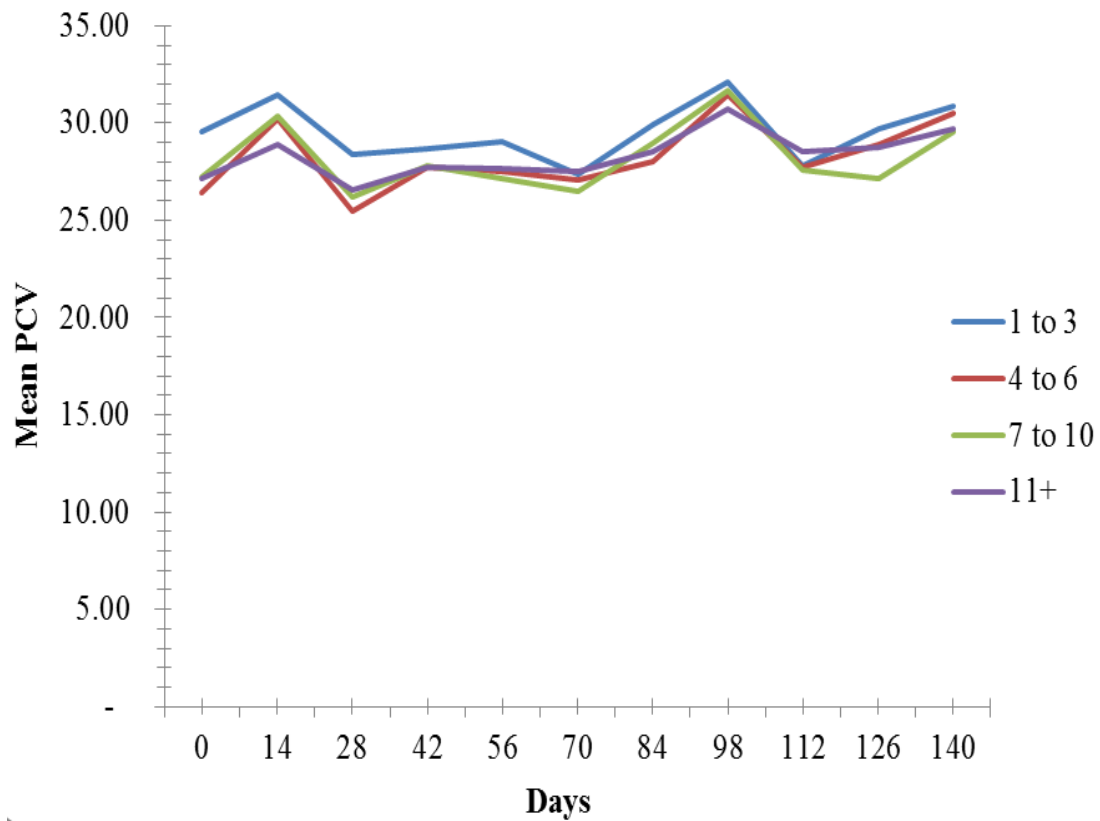
Figure 4: PCVs for all animals throughout the trail period and their age (months) categories.

4.5.2 EPGs trends during the trial period

Results on the trend of EPG in male and female animals during the trial period is summarized in figure 5. It was observed that the EPG of male animals was significantly ($P<0.05$) reduced gradually and overall the reduction was more in male animals compared to females. For all age categories, EPGs dropped from day 0 to day 42 and rose again with highest EPG recorded on day 126 before dropping back. Overall trend of EPGs in both sheep and goats (combined) is shown in figure 6. It was found that the EPG deceased gradually after vaccination until post-vaccination then started to rise again. On day 84 and 112, the EPG dropped with increase in between the days and lastly gradually falling to the last day.
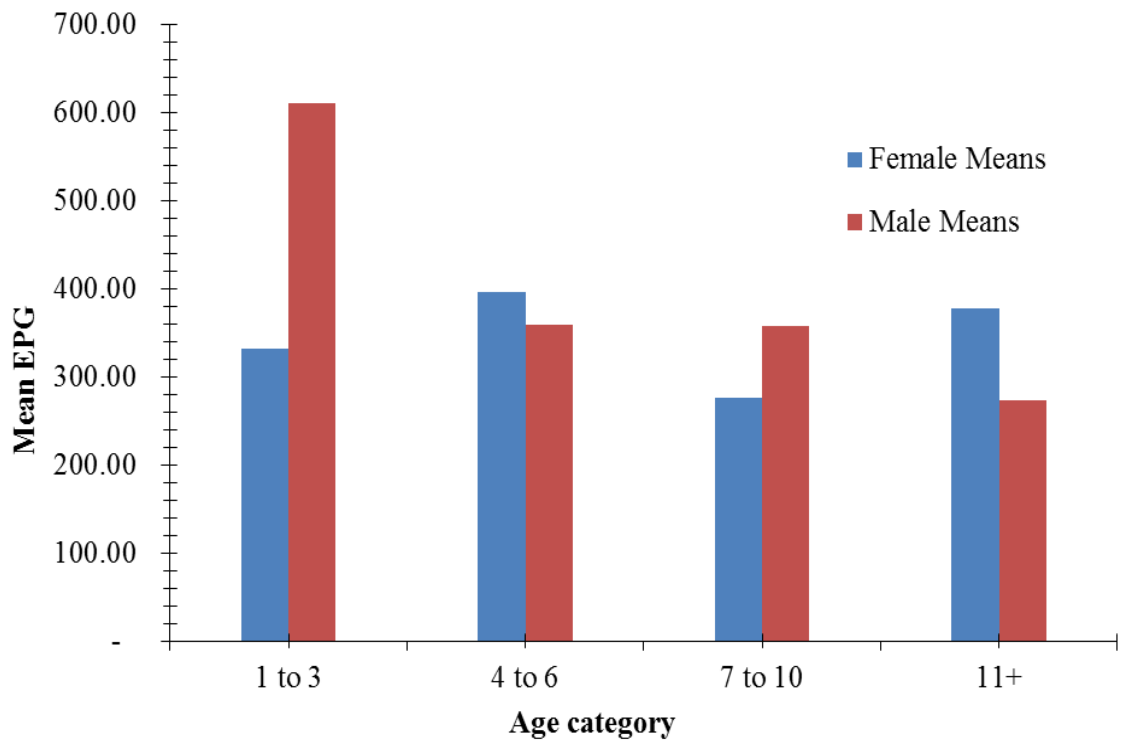
Figure 5: Overall mean EPGs for male and female animals categorized by age in months during the trial time
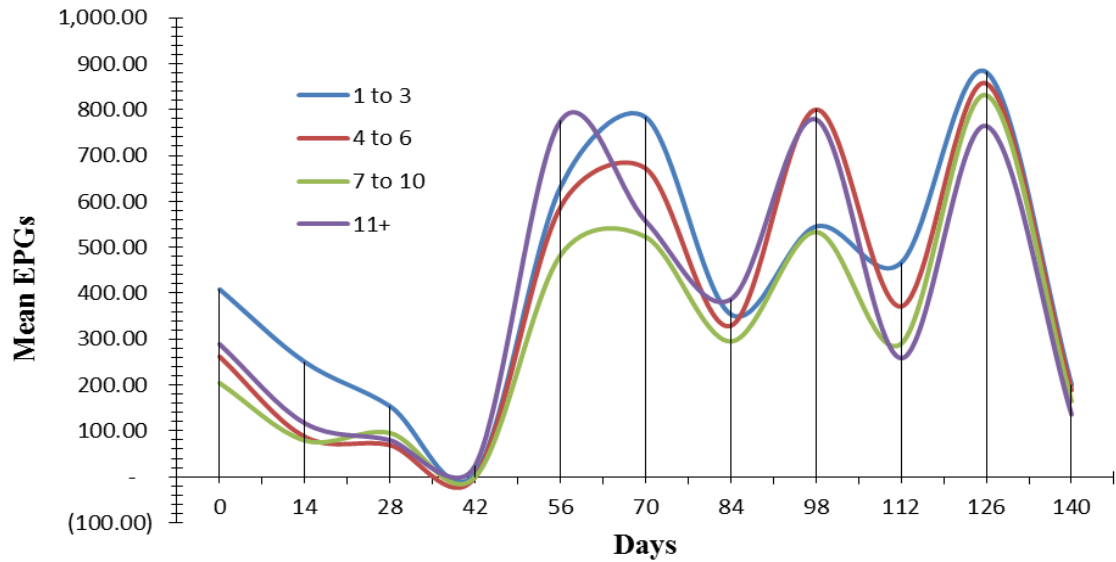
Figure 6: Overall mean EPGs of sheep and goats stratified by days

4.5.3 Trend of larvae in vaccinated and control animals

The trend of larvae counts in vaccinated and control animals is shown in figure 7. It was observed that the mean number was significantly higher in control group than in vaccinated goats throughout the trial period ($p<0.05$).
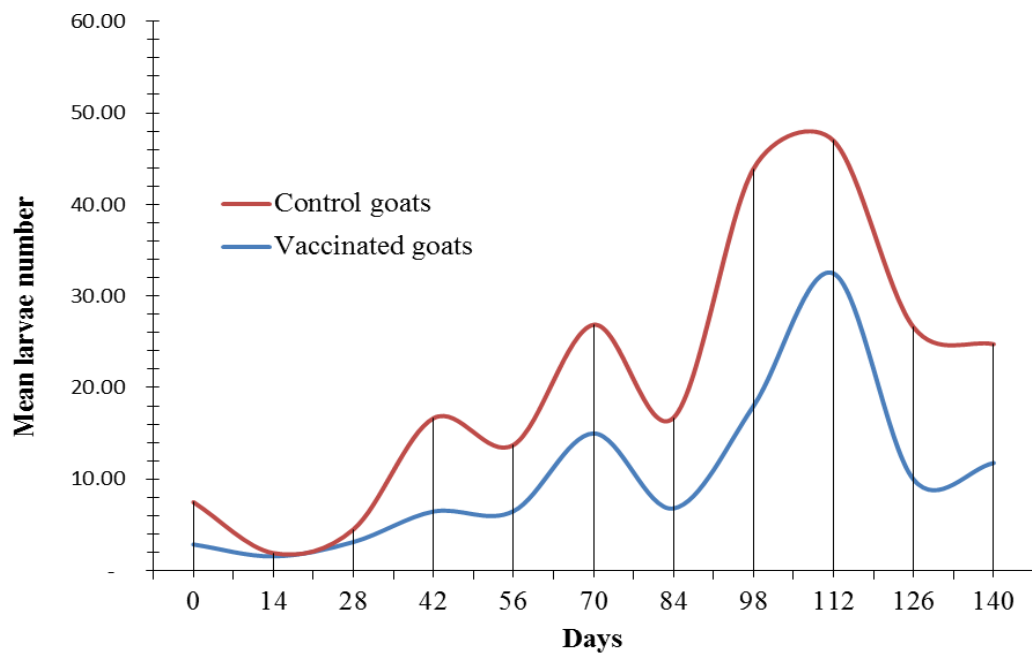
Figure 7: Mean number of larvae for control and vaccinated goats in Melela-Mlandizi

CHAPTER FIVE

5.0 DISCUSSION

This study established the status of gastrointestinal nematodes and coccidia parasites in sheep and goats flocks in Mlandizi village of Melela ward in Mvomero district. The proportions of goats and sheep infested with worms were 54.0% and 67.8% respectively while those infested with coccidia was 52.7% and 49.2% respectively. The lack of clinical signs coupled with the fact that egg and oocyst counts were low indicated that both types of infestations were of low grade. The findings in the current study are of relatively low proportins compared to those reported by Fakae (1990) who found high prevalence of *Haemonchus contortus* (87.1%) compared to 50.7% recorded in this study. Another study by Welemehret *et al*. (2012) found that the overall prevalence of nematodes was 56.25% and 35.33% in sheep and goats, respectively. This is low for sheep and high for goats as compared to this study. The study by Welemehret *et al*. (2012) also found that strongyles were the most prevalent parasites as in this study.

Among the stongyle larvae species identified, *Haemonchus spp.* was the most prevalent (50.7%). The Maasai do not normally deworm their stock routinely (Roderick *et al.,* 2000), offering an explanation for the relatively high proportion of worm infested animals in the study area. Adult sheep and goats do not develop strong immunity to *Haemonchus spp.* (Whittier *et al.,* 2009; Alba-Hurtado and Muñoz-Guzmán, 2013) and, if not treated with anthelmintics, will continue to shed eggs, thus contaminating the pasture and maintaining the life cycle.

The proportion of sheep infested with helminths was significantly higher (67.8%) than what was recorded in goats (54%). These findings tally with those of Ayana *et al.* (2009), who suggested the disparity could emanate from differences in the rate of acquisition of natural immunity and feeding habit. Goats, being browsers by nature only graze when shrubs and bushes are unavailable, thereby reducing the risk of being infected with 3rd stage larvae on the pasture.

During this study when identifying strongyle eggs, *Eimeria* oocysts were detected as well. This was interesting as studies shows that goat and sheep infected by strongyles are likely to be infected by *Eimeria* as well. Coccidiosis is one of the most economically and most clinically important protozoan diseases of goats and sheep in sub-Saharan Africa and commonly occurs with helminth infestation (Kusiluka *et al.,* 1996). The proportion of goats and sheep infested with coccidia was 52.7% and 49.2% respectively. The coccidial infestation recorded in this study was low compared with what was reported by Girma *et al*. (2016) where the parevalence was 66.9% and 62.7% in goats and sheep, respectively. Low concentrations of oocysts in the faeces indicated the presence of low grade coccidial infestation at Mlandizi village as might be expected for animals grazed extensively. The Maasai graze their young kids and lambs near their traditional houses, far from the adult animals, thus reducing exposure to heavy pasture contamination (Roderick *et al.,* 2000). Interestingly, the goats did not shed as many oocysts as the sheep, possibly because they browse rather than graze and thus are less likely to ingest oocysts on the ground. The Maasai do not normally use coccidiostats and so their kids and lambs which have the highest oocyst counts continue to contaminate their environment, but,

thanks to the development of immunity, clinical disease occurs rarely in adults (Radostits *et al.,* 2006).

In this study, *E. caprina* (21%) was the most frequently encountered species in goats, followed by *E. parva* (16.2%). This is inconsistent with the finding of Gul and Değer (2002) who found *E. parva* to be the most frequently encountered species in sheep. The study revealed that 32.2% of the sheep were shedding *Eimeria* oocysts, significantly less than the equivalent figure for the goats and contrasted with the findings of Waruru *et al.,* (2005). *Eimeria* oocysts were detected more commonly in males than females of both species. Similarily Gulland and Fox, (1992) reported that except during lambing the proportion and intensity of infection (faecal oocysts) was higher in males than females, but decreased with age.

Oocysts were also more prevalent in lambs than older sheep corresponding to the findings of Balicka-Ramisz (1999); Waruiru *et al.* (2005) and Ayana *et al.* (2009), and suggesting that immunity was acquired with age. The results indicated that both helminth and coccidial infections are present in traditionally managed small ruminants' flocks and it is possible that the effects of this combination may have some impact on productivity. The Packed Cell Volumes (PCVs) remained above 25% for both sheep and goats. This compares well to studies by Besier *et al.* (2012) where the comparative *H.contortus* egg count differences were not as dramatically reflected in the measures of anaemia, as mean PCVs of both control and vaccinated groups declined by to a similar degree over the trial period.

The mean number of strongylid larvae between vaccinates and controls goats was statistically significant ($p<0.05$) for ages 3 and 9 months in which the mean number of larvae after faecal culture were lower in vaccinated goats than in the control (unvaccinated goats). This is the the effect of the vaccine reducing the number of larvae for the vaccinated animals. Studies by Verveldei *et al.* (2001) showed that experimental challenge infection by immunization with excretory/secretory products (ES) from *Haemonchus contortus* depends on the age of the sheep. It was further found that no reduction in worm burden was found in 3-month-old lambs, and the responses measured in young lambs were similar to the responses in sheep, but the height of these responses was in general of a lower magnitude.

The egg counts were quite low throughout the trial, with group means nearly always below 1000 total EPG and *Haemonchu*s specific counts about half that. Generally, young animals (age 2-5 months) had relatively higher EPGs than older animals. The overall mean EPGs revealed a significant difference for both sheep and goats and both sex ($p<0.05$). No differnces in either total or *Haemonchus* specific egg counts were observed between vaccinates and controls after third vaccination in either sheep or goats. This observation was different fom findings by Besier *et al.* (2012) who reported protective response in terms of both the reduced development of worm burdens from larval intake and a significant anthelmintic response, seen as a sharp fall in worm egg count after vaccination. The reasons for the observation of the vaccine effect may emanate from low infection rates as effects of vaccine is not well established with small EPGs. The average eggs per gram (EPGs) in goats were 276 and the outputs ranged from 100 to 2,100. In sheep the average EPG was 307, with

egg outputs ranging from 100 to 1,900. These EPG mean counts were relatively low to give a strong statistical difference between vaccinates and controls. Other factors could be few numbers of eggs leading to few numbers of larvae and low hatchability as a result of poor viability of eggs that could be due to immune factors of the animals. Furthemore, another reason might emanate from misidentification of larvae and *Haemonchus* eggs as morphological features only were used.

Identification is challenging as worms looks alike and are not straight forward identified just as studies by van Wyk and Mayhew, (2013) showing that the only practical method available to the helminthologist for obtaining an indication ante-mortem of the worm genera with which cattle and small ruminants are infected is to identify the larvae that are found in fresh faeces or that develop in cultures of the faeces of the animals. However, even though the infective larvae (L_3) of the common worm genera are generally more easily identified than the ova, even this is often feasible only for the experienced person; distinguishing features such as the shape of the "head" (cranial extremity) of the larva or the length of the sheath "tail" (the extension of the sheath from the tip of the larval caudal extremity to the tip of the tail of the sheath, are similar to all but the practiced eye. While the first-stage larvae (L_1) of protostrongylids or the third-stage larvae of strongyles (L_3) can be measured, it is not practical to measure each larva when doing routine differential diagnostic counts. On the other hand, there are possibilities of immunologically "non-responder" sheep which may not portray good results as reported by Besier *et al.* (2012). Further investigations are required in different environments and under different rates of *H.contortus* challenge in our country. Fluorescent microscope technique has to be

applied to identify well *Haemonchus contortus* eggs and its Larvae stage 3 (L_3) and quantify well the EPG reducing capacity of Barbevax® in our country to increase productivity of small ruminants and improve livestock keepers livelihood.

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

The study revealed that gastrointestinal parasites are prevalent in Melela-Mlandizi areas and *Haemonchosis* being the leading infestation affecting small ruminant flocks in traditional settings. Others were *Trichostrongylus* spp., *Oesophagostomum* spp., *Cooperia* spp. and *Strongyloides* spp. *Moniezia* was also detected. The presence of coccidian oocyst as well indicates that coccidiosis is a problem though the OPGs were low. The most prevalent was *Eimeria caprina*; others were *E. granulosa*, *E. parva*, *E. arloingi*, *E. christenseni*, *E. marsica* and *E. ovinoidalis*. The clinical disease manifestation of helminth and coccidioa infection may be related to any disease sharing signs known by the owners and diagnosis is confirmed by EPGs and OPGs analysis. When infection rates are low, clinical manifestation is not easily noticed. The effect of the vaccine on helminth infestation was the reduced EPGs after first and second vaccination for both sheep and goats. The mean number of larvae for vaccinated goats remained lower than control goats throughout the trial period.

6.2 Recommendation for future work

The control of helminth and coccidia is of great importance in saving animal lives and welfare and increasing their productivity and income to the livestock keepers. Despite the low prevalence observed, the proper use of anthelmintics is still of value for helminths. Coccidiostats on the other hand should be used by livestock keepers.

Deworming all the stock may be done at every 3 months as a herd health approach and a regular consultation with veterinarians should be done as additional care and attention may be adviced accordingly. The use of the vaccine for haemonchosis is highly advised in our country as it wil be easy to use and cost effective compared to frequent use of anthelmintics and their resistance. This work ended with coprological analysis only and in future serological analysis will be of greater value in order to know how the antibody-antigen reactions differs between vaccinates and control animals. Further more, the trial may be done in the two seasons; the rainy and dry seasons to see how the vaccine performs in the two seasons with variability in worms' burdern.

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APPENDIX

Appendix 1: Distribution of animals and their copro culture groups in each flock

Flock	Label	Status	How many	Total
	1	GV	10	
	2	GC	4	
1	3	SV	5	19
	4	GV	2	
	5	GC	5	
	6	SV	3	
2	7	SC	4	14
3	8	GC	2	2
	9	GV	4	
	10	GC	3	
	11	SV	5	
5	12	SC	6	18
	13	GC	2	
	14	SV	3	
6	15	SC	2	7
	16	GV	2	
	17	GC	1	
	18	SV	5	
7	19	SC	5	13
	20	GV	2	
	21	GC	6	
	22	SV	4	
8	23	SC	4	16
			Grand total	**89**

KEY: GV= Goats vaccinated, GC= Goats Control, SV=Sheep Vaccinated,

GC=Sheep control