# PREVALENCE OF GASTROINTESTINAL PARASITES, ANTHELMINTIC USE AND RESISTANCE IN SELECTED SHEEP FARMS IN KASARANI, NAIROBI COUNTY.

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REGNO: J56/36188/2019

A THESIS SUBMITTED IN PARTIAL FULLFILMENT OF THE REQUIREMENT OF THE MASTERS OF SCIENCE DEGREE OF THE UNIVERSITY OF NAIROBI.

(APPLIED VETERINARY PARASITOLOGY)

DEPARTMENT OF VETERINARY PATHOLOGY, MICROBIOLOGY AND PARASITOLOGY

FACULTY OF VETERINARY MEDICINE

UNIVERSITY OF NAIROBI

DATE: 8th March 2022

**DECLARATION**

This thesis is my original work and has not been presented for a degree in any other university.

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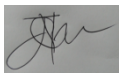
   Signed……  ……date……8/3/2022……

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## DEDICATION

I dedicate this piece of work to my lovely mother Sarah Tangus, my lovely husband Langat Robinson, my sons Ryan Kipchumba and Rollins Kipkorir and my daughter Shantel Chelangat.

**ACKNOWLEDGEMENT**

First and foremost, I thank God for granting me his grace, favour and good health throughout the research exercise. I would like to acknowledge my supervisors, Prof. Chege, Dr. Karanja and Prof. Gachuiri for their unconditional support and all their contribution from beginning to the completion of this work. I thank my family for their spiritual, motivational support and encouragement. My gratitude also goes to Dr. Karugu the county director of veterinary services, Nairobi County for permitting this work to be done in Kasarani sub-county and for allowing his officers (Ms. Barasa Judith and Ms. Joyce Mwikali Ndunda), to take part in the project.

Besides I would like to thank the farmers, veterinary service providers, agricultural and veterinary drugs stores owners and salespersons in Kasarani Sub-County for their co-operation throughout the research period. I also thank my colleagues, Dr. Hellen Maingi and Mr. Ephantus Nyaga for their unconditional support throughout the exercise. I thank the Parasitology Laboratory technologists, Ms. Beatrice Mutende and Ms. Edith Keya for their assistance during parasitological processing of data.

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## ABSTRACT

The adverse effects of gastrointestinal parasitic infection have resulted in low productivity of sheep in Kenya. As a result, farmers relied on anthelmintic therapy that may lead to occurrence of anthelmintic resistance. The occurrence of clinical and sub-clinical cases despite treatment, enhanced the need to determine prevalence and intensity of gastrointestinal parasites infection in sheep, assess knowledge, practice and attitudes towards treatment and control of helminthes infection in sheep and test for anthelmintic resistance.

A cross-sectional study was conducted in 30 selected sheep farms in Kasarani to determine the prevalence and intensity of gastrointestinal parasites in different breeds of sheep. One thousand six hundred and forty-two (1642) faecal samples were subjected to coprological examination to determine the egg per gram (EPG), oocyst per gram (OPG) of faeces with a detection level of 100 EPG/OPG and for the presence of cestode and trematode eggs. The overall prevalence of gastrointestinal parasites was 99.6 %. The prevalence of strongyle eggs was 72%, coccidial oocyst 49% and 7% for tapeworm eggs. There was no significant difference in prevalence of strongyle between adults and lambs (P-value 0.7), males and females (P value 0.4) and coccidial infection between adults and lambs (P value 0.2) and male and female (P value 0.3). Prevalence of tapeworm infection in between adults and lambs (P-value 0.000) and male and female (P value 0.0001).

The overall intensity of strongyle (480 mean egg count (MEC) and coccidial (330 mean oocyst count (MOC) infection in sheep were low. At farm level the intensity of strongyle (32-1561 MEC) and coccidia (7-2034 MOC) ranged from mild to severe (MEC, 2034 MOC). Helminthes spectrum showed 90% *Haemonchus* species, 5% *Trichostrongylus* species and 5% *Oesophagostomum* species. There was no evidence of trematode infection in the sampled sheep.

Survey on knowledge, practice and attitude towards treatment and control of gastrointestinal parasites showed that all the respondents 77(100%) were aware about helminthes infection in sheep. Classes of anthelmintics available in veterinary drugs stores were benzimidazole, imidazothiazole and macrocyclic lactones. The proportions of farmers using these anthelmintics were 43%, 30% and 27% respectively. The ease of administration (70%) and price (30%) greatly influenced the choice of anthelmintics to use. Veterinary consultation was rare (13%) in suspected case of sheep helminthosis. Dose determination was based on visual estimation of the sheep's weight (67%) and weight of individual sheep (33%). Deworming was done every three months (67%) and whenever there was sign of helminthes infection (33%). In every subsequent treatment a different class of anthelmintics was used (73%).

Faecal egg count reduction test (FECRT) detected multiple anthelmintic resistance whereby resistance to albendazole, levamisole and ivermectin was confirmed in one farm with FECR% of 11.1%, 59.3%, 59.3% respectively. More farms (60%) showed resistance to albendazole than to levamisole (40%) and ivermectin (40%). *Haemonchus contortus* and *Trichostrongylus* species were resistant to the three anthelmintics while *Oesophagostomum* species were resistant to levamisole and ivermectin.

This study demonstrated high prevalence of 99.6% of gastrointestinal parasites infection in sheep. The inappropriate dosing, frequent deworming and short alternation period might have resulted in occurrence of multiple and multi-generic anthelmintic resistance (AR) in sheep in Kasarani Sub-County. Therefore, there is need to educate farmers on proper use of anthelmintics, the risk of anthelmintic resistance occurrence and its consequences on production.

# CHAPTER 1: INTRODUCTION AND OBJECTIVES

## 1.0 INTRODUCTION

Sheep have a high potential to affect the socio-economic growth of most African rural communities. Improving sheep production can increase farmers' income, ability to acquire more inputs for other production activities, hence improved standard of living. Sheep of different breeds are mainly kept for mutton, milk, wool or dual purpose. Common breeds suitable for different regions in Kenya include: Merino, Hampshire down and Corriedale that are kept in high altitude areas; East African fat tailed type and dorper that are kept in medium altitude areas. East African fat-ramped type is kept in both high and medium altitude areas while red Maasai, dorper and Persian black head are kept in low altitude areas (Baker *et al,* 2002).

Sheep population in the world is approximately 1.2 billion (Mazinani and Rude 2020). In Africa sheep population is 352 million representing 30% of the world's population of small ruminants (Mazinani and Rude 2020). Kenya's small ruminant population is estimated to be 46 million out of which 42% are sheep (FAOSTAT, 2020). The livestock sector in Kenya is a source of livelihood to rural and urban population and contributes 12% of Gross Domestic Product (GDP) (Ministry Livestock and Fisheries, 2008). In Nairobi, 10% of the livestock population are small ruminants consisting of approximately 4%, sheep. In Kasarani Sub-County 3% of the livestock population are sheep (KBS, 2019).

In arid and semi-arid land (ASALS) livestock production industry is estimated to contribute 90% of employment and 95% of family income (FAO, 2018). Sheep are kept in ASALS since in times of drought they can easily be de-stocked and re-stocked afterwards thus reducing production cost due to starvation (FAO, 2018).

Sheep grow at a faster rate, with high reproductive capacity and can withstand harsh environmental

conditions, yet require minimum cost of production (Stephen, 2017). Therefore, there is need to increase sheep number and productivity through improved breeding, proper nutrition and management of production limiting diseases. Parasitic infections especially gastro-intestinal (GI) parasites are among the major production limiting diseases. Blood sucking nematode *Haemonchus contortus* and *Eimeria* species and *Cryptosporidium* species are the most important helminthes and coccidia respectively (McRae *et al*., 2015).

Production losses caused by these gastrointestinal parasites are manifested by mortality of up to 40%, reduction in milk, wool and live weight (up to 50 %). Besides, their higher susceptibility to GI parasites necessitates heavy reliance on anthelmintic therapy by farmers which has resulted to anthelmintic resistance (AR) (McRae *et al.*, 2015).

Gastrointestinal parasites limits sheep productivity, farmers tend to rely on anthelmintic therapy resulting to occurrence of anthelmintic (Charlier *et al.*, 2014). Hence the need to test for prevalence and intensity of these parasites, assess knowledge, practices and attitudes towards treatment and control of gastrointestinal parasites in sheep and test for anthelmintic resistance (AR) in selected sheep farms in Kasarani Nairobi County, Kenya.

## 1.1 OBJECTIVE

Investigation of the prevalence of helminthes and coccidial infestation, anthelmintic usage and anthelmintic resistance in selected sheep farms in Kasarani, Nairobi County.

## 1.2 SPECIFIC OBJECTIVES

1. To determine the prevalence, intensity and spectrum of helminthes and coccidial infestation in sheep
2. To assess knowledge, practice and attitude of farmers, veterinary service providers and agricultural and veterinary drugs sales persons towards control of helminthes and coccidia infestation in sheep.
3. To investigate the occurrence of anthelmintic resistance.

## 1.3 RESEARCH QUESTIONS

1. What is the prevalence of gastrointestinal parasites of sheep in the study area?
2. What are the farm level practices and farmer knowledge on use of anthelmintics in sheep flock?
3. Which anthelmintics has the parasites develop resistance to?

## 1.4 HYPOTHESIS

It is hypothesized that gastro-intestinal parasites infections in sheep limit their productivity hence inappropriate use of anthelmintics which result to anthelmintic resistance and presence of clinical and sub-clinical cases despite treatment.

## 1.5 JUSTIFICATION

Gastrointestinal parasitoses cause high mortalities, reduction in live weight and reduced overall sheep productivity. Determination of prevalence of gastrointestinal parasites is important in order to account for their impact in sheep production. Inadequate awareness of helminthes, their effects on sheep production, treatment and control is the knowledge gap that exists among sheep farmers in developing countries. Therefore, the need to assess knowledge, practice and attitude towards control and

treatment of sheep's helminthes. Anthelmintic resistance is a major challenge in the sheep production industry and it's attributed to inappropriate anthelmintics usage hence the need for resistance testing. Sheep production is an importance social-economic activity for farmers in Kasarani sub-county. However adverse effects of gastrointestinal parasites limits sheep productivity which make farmers to rely on anthelmintic therapy which led to occurrence of anthelmintic resistance. Therefore, this study becomes relevant as it focuses on occurrence and levels of helminthes infection and resistance to anthelmintics and contributing factors.

# CHAPTER 2: LITERATURE REVIEW

## 2.1 Sheep Farming Systems

The three main sheep farming systems that are Practised worldwide are extensive, intensive and pastoralism (Stephen, 2017). Emerging sheep production are currently under way especially in china where lambs are kept inside the house with their mothers for life while in USA, Australia and Mexico lambs to be slaughtered are confined in a feed lot and given feeds with high energy (Stephen, 2017).

### 2.1.1 Extensive Production System

There are many extensive sheep production systems in the world. The sheep are kept on pasture throughout the year, with no housing, no supplementation and enough Behavioral freedom. Lamb mortality, inadequate nutrition, gastro-intestinal parasitism, anthelmintic resistance and market access are major production limiting factors (Stephen, 2017).

### 2.1.2 Intensive Production System

This is mainly a dairy system and is practice in Europe, Middle East and Near East. Dairy ewes produce a maximum of 600 litres of milk per lactation which is used for cheese, yoghurt and ice-cream production. Early weaning of lambs, housing and diseases are the main welfare problems in this system (Stephen, 2017).

### 2.1.3 Traditional Pastoralism Production System

This system is practice in dry areas where climatic condition is uncertain and decrease in rainfall increases the economic dependent of livestock (Stephen, 2017). Traditional pastoralism is categorized into nomadic, transhumance and agro-pastoral depending on depending on the degree of movement of animals (FAO, 2001). Nomadic pastoralists can't settle in one place as they move with their animals from one location to another depending on seasonal changes. Transhumance, describe pastoralists who move their flocks within a certain locality while utilizing pasture available during a

given season, while agro-pastoralism describes pastoralists who have already settled in one place where they are able to cultivate sufficient land to provide for their families and keep enough livestock that can be grazed near their homestead or village. Agro-pastoralists own large flock of sheep that are sent to graze in range lands by nomadic shepherds. Forage availability is the main challenge in traditional system since climate is unpredictable. In arid areas lack of adequate water, diseases and attack by predators hinders production and good animal welfare standards (Stephen, 2017).

In Kenya 80% of small ruminants are raised in arid and semi-arid area where they serve as an important source of dietary proteins in terms of milk and meat and it's the best choice of enterprise in this area (Githigia, 2000). In this area extensive production system prevails where sheep are kept in permanent meadows and range land pastures (FAO, 2017). Communal grazing with high stocking rates, reflect low production rates, high mortality, decreased weaning rate and little returns. However, challenges limiting this production system include; inadequate nutrition, lack of proper housing, diseases, parasites and other health conditions (Kosgey *et al.,* 2008). Pastoralist communities in medium potential areas have been changing to agro-pastoral system whereby they integrate crop production and make it their main source of income (Kosgey *et al.,* 2008).

Mixed farming is practice in irrigated and medium to high rainfall regions in Kenya where various food and cash crops are integrated with few livestock. Sheep production is done in medium or small scale mixed with other livestock. Sheep are mainly kept for family and provide agricultural input (manure) needs rather than a business enterprise (Kosgey *et al.,* 2008).

Smallholder sheep farmers in Kenya have a maximum flock size of 66 mixed breeds while pastoral/extensive farmers have a maximum flock size of 914 indigenous sheep and most farmers in each system keep sheep for regular cash income or as insurance in emergency situation (Kosgey *et al.,* 2008). In Nairobi county small scale farms keep 1-5, medium scale farms keep 10-20 and large-

scale farms keeps 50-500 small ruminants and sheep breeds kept are mainly red Maasai, dorper and mixed breeds (Alarcon *et al.,* 2017). Challenges faced by sheep and goats' farmers in Nairobi are; inadequate grazing fields, theft and diseases ((Odoi *et al.,* 2000). Constrains to sheep production systems include; parasites and disease, breed and breeding management, inadequate nutrition, socio-economic aspects, drought and predators (Odoi *et al.,* 2000).

### 2.2 Parasitism in Sheep

Sheep are affected by both external and internal parasites which limit their productivity. External parasites feeds on body tissues (blood, skin and hair) causing wounds, irritation, discomfort and transmit diseases. External parasites include: *Hematophagus* insects (black flies, fleas, M*elophagus*, midges, mosquitoes, tsetse-flies), non-biting insects (lice, houseflies), insect larvae causing myiasis (blowflies, nasal botfly, screw worm flies, *Dermatobia*), ticks (*Amblyomma, Dermacentor, Haemophysalis, Hyalomma* and *Ixodes*) and *Psoroptes* and *Sarcoptes* mites. Internal parasites include; helminthes and are grouped into; nematodes, trematodes and cestodes and coccidia (*Eimeria* and *Isospora*) (Soulsby, 1982; Hansen and Perry, 1994).

### 2.3 Gastrointestinal parasites of sheep

#### 2.3.1 Helminthes parasites of sheep

Helminthes and coccidia are the major gastro-intestinal parasites of sheep. Helminthes parasites of sheep belong to class Nematoda, Trematoda and Cestoda. These helminthes are located in different parts of the GI tract and other organs and tissues. *Trichostrongylus axei* and *Haemonchus contortus* are located in abomasum while *T. columbriformis, T. vitrinus, T. rugatus N. spathiger, Strongyloides, Cooperia, N. felicolis, Bunostomum, Monenzia expansa* and *M. benedeni* are located in the small intestines. In the large intestines are *Trichuris ovis, Oesophagostomum venulosum* and *Charbatia ovina. Protostrongylus rufescens, Dictyocaulus filaria* and *Muellerius capillaris* are located in the

lungs. Sheep trematodes includes; *Fasciola hepatica* and *Paramphistomum* are located in the liver and rumen respectively. Cyst forming helminthes *Cysticercus ovis* and *Cysticercus taenucolis* are located in muscles tissues and abdominal cavity (Kym, 2018; Foreit, 1999; 2001).

Effect of helminthes parasites are sub-clinical or chronic in nature and might not be detected Soulsby (1982); Urquhart *et al.,* (1996), causing high economic loss. Among the nematodes *Haemonchus contortus* is the most pathogenic as it's a blood sucker causing severe anaemia in acute cases, it causes severe blood and protein loss into the abomasum and intestines resulting in edema (Abebe *et al.*, 2018). Trematode infection depend on availability of intermediate host (IH) with favorable climatic and ecological conditions for IH (Sissay, 2007).

An outstanding sign of gut parasites infection is a dull sheep with anorexia, poor body condition, lag behind the flock and diarrhoea in severe case. Young and malnourished sheep, lactating and pregnant ewes are highly susceptible to git parasites infection. In Kenya periods of cold and wet condition are high risk time for git parasites infection especially helminthes infestation (Baker *et al.*, 2002). Worms become a problem if their number causes production losses and increases sheep susceptibility to other disease.

#### 2.3.1.1 Factors that influence level of host infection

Increase rate of pasture contamination with gastro-intestinal parasites' infective stages, host becoming highly susceptible to infections due to weak immunity, entry of these susceptible stock to a contaminated field, failure to completely get rid of gastrointestinal parasites from an infected host, application of less effective anthelmintics and inability of anthelmintics to effectively clear the infection due to emergence of resistant parasitic worms are the main factors that enhance level of host infection (Hansen and Perry, 1994; Ng'ang'a, 2002; Sissay, 2007). Besides, livestock production

system influence transmission and accessibility of infective stages of gastrointestinal parasites for example extensive systems have low concentration of infective stages unlike in overstocking where there is increased contamination of pastures (Ng'ang'a, 2002). Besides, young sheep less than 12 months old are more susceptible to parasitism with high worm burden and excrete high number of eggs in faeces than adult sheep.

Periparturient rise (PPR) in worm egg count of lactating and late pregnant ewes goes up 6-8 weeks soon after lambing. PPR is associated with high worm burden, high ewe death rate, increase pasture contamination and hence high infectivity of lambs grazing in the contaminated field (Ng'ang'a, 2002, Ng'ang'a *et al.,* 2006). PPR occurs due to temporary reduction in immunity affected by lambing period, current ewe worm burden, pasture quality and quantity, contamination level with infective larvae, ewe's genotype, age and parity (Kym, 2018; Ng'ang'a *et al.,* 2006).

#### 2.3.1.2 Nematode Life Cycle

Most important nematodes of sheep share the same life cycle which is simple direct with no intermediate host except lung worms, *Protostrongylus rufescens* and *Muellerius capillaris* whose infective stage development occurs in snails, slugs and snails respectively (Hansen and Perry 1994; Kym, 2018. Once the eggs are passed out in faeces development occurs immediately provided the temperature is suitable with sufficient oxygen and moisture. At $26^0$c larval stage one hatch within 24 hours, but at lower temperatures with anaerobic condition hatching takes quite longer. Development of egg to larval stage three takes 4-6 days at $27^0$c with enough oxygen supply and moisture (Kym, 2018; Soulsby, 1982; Urquhart *et al*., 1996).

Among the most important genera of sheep, *Haemonchus contortus* is the most fecund (5000-10000 eggs per female per day), then *Chabertia ovina* and *Oesophagostomum venulosum* (3000-5000

eggs/female/day) and *Trichostrongylus* species is the least fecund (100-200 eggs/female /day). Inside the egg development takes place from the original cell to a morula and to larva, which moults from larval stage one to larval stage five ($L_1$-$L_5$). $L_1$ to $L_3$ are free living stages on pasture while $L_4$ and $L_5$ are parasitic stages. At each moulting stage the cuticle is shed and a new one develops with the exception of $L_3$ which retain the sheath till its ingested by the host for ex-sheathment to occur (Kym, 2018; Soulsby, 1982; Urquhart *et al*., 1996).

Free living Larval stage one ($L_1$) and larval stage two ($L_2$) actively feed on bacteria, infective stage larval stage three ($L_3$) are inactive, if larval stage three ($L_3$) are free living they retain the cuticle of $L_2$ which act as a protective sheath except in *Strongyloides papillosus*. En-sheathed larval stage three ($L_3$) doesn't feed and once the energy reserves are depleted they die. Sheep get infected through the ingestion of infective stage larval stage three ($L_3$) in pasture, *Bunostomum* species can also infect through skin penetration with a pre-patent period of 8-10 weeks while in oral infection it takes 17-21 days.

Survival of free-living stages of nematodes depends on the availability of environmental temperature and moisture. Eggs are very lethal to desiccation but $l_3$ have a protective sheath, which make them more resistant to desiccation for example larval stage three ($L_3$) of *Trichostrongylus* species. $L_3$ of *Haemonchus contortus* Are active but more vulnerable to adverse environmental conditions than other major strongyle (Kym, 2018; Priyanka *et al.*, 2020).

#### 2.3.1.3 Effect and clinical signs of GI nematode infection

Gastro-intestinal nematodes induce an inflammatory reaction in the gut, severity of which depends on the number of parasites. The resultant effect of this is severe scouring and faecal soiling. The patho-physiological effects of GI nematodes include; inappetance (decreased food intake), alteration

of gut normal function (protein loss, increase gut motility, increased git secretion, impaired digestion and nutrients absorption) and anaemia due to *Haemonchus contortus* infection.

Adult *Haemonchus contortus* can suck 0.08 ml of blood from the abomasal mucosa in a day. This result in loss of 2-3% of the total blood volume per day hence anaemia, stunted growth and high mortality rates exceeding 30 %. Anaemia is manifested by pale mucous membrane, reduced capillary refill time in acute and sub-acute cases and sub-mandibular edema in chronic cases (Hansen and Perry, 1994). *Trichostrongylus* species. Causes diarrhoea, reduced wool growth, faecal soiling, wool breaks and high mortalities (Kym, 2018).

The initial development of *Oesophagostomum venulosum* larva occurs in small intestines causing pimply gut or knotty gut which are fibrous nodules with thick pus (greenish) in the mucosa and muscular layer of small intestines. Later causes severe caseous lesions in caecum and peritonitis (Soulsby, 1982; Urquhart *et al.*, 1996).

**2.3.1.4 Life cycle of sheep's trematode**

Adult *Fasciola hepatica* is leave-like in shape and measures 30x30 mm found in the bile duct, it's hermaphroditic in nature, producing eggs that are expelled into the intestines with bile and shed in faeces. The eggs embryonate and hatch into miracidia in presence of moisture (water or wet pasture), suitable temperature, adequate oxygen supply and light.

The ciliated miracidia actively seek and penetrate a suitable intermediate host, snail of genus lymnaea, where it undergoes further development (asexual multiplication) into sporocyst and finally cercaria. Five to seven weeks later cercaria emerge from the snail and actively swim to encyst on herbage and loses its tail to form metacercaria (infective stage) which is relatively resistant and can survive adverse environmental condition.

The final host gets infected through ingestion of contaminated herbage with metacercaria (Hansen and Perry, 1994). In the host's intestines, the young flukes excyst and penetrates walls of the intestines and to the liver parenchyma through the abdominal cavity and liver capsule. The immature flukes wander through the liver tissues for weeks causing serious pathology.

They later reach their predilection site (bile duct) where they mature and eggs production begins. Liver flukes have a high egg producing capacity (5000-20000 eggs/day) and pre-patent period for *Fasciola hepatica* is 8-12 weeks (Hansen and Perry, 1994).

**2.3.1.5 Life Cycle of Paraphistomes spp.**

Paraphistomes are gastrointestinal trematodes that are maggot-like worms that are thick, fleshy and short (4-12mm). Its life cycle is similar to that of *Fasciola*, water snail is the intermediate host where the asexual multiplication take place (*Miracidia- sporocyst- cercaria*). *Cercaria* encyst in herbage and loss its tail to become the infective stage metacercaria.

Host animal get infected through ingestion of encysted *metacercaria* in pasture which excyst in duodenum releasing young flukes. The young flukes attack and invade the proximal 3m of the gut. 10-30 days later the immature flukes re-emerge from the intestinal mucosa, moves toward the rumen and reticulum where they attach onto the mucosa and mature into adults and start egg production. Paraphistomes have a pre-patent period of 70 days. They are reddish in appearance in between the papillae of rumen and reticulum (Hansen and Perry, 1994).

**2.3.1.6 Life Cycle of Sheep's Cestodes**

*Moniezia expansa* and *Moniezia benedeni* are main cestodes of sheep and they are located in the small intestines. They have indirect life cycle with oribatid acting as the intermediate host which lives in soil. They dwell on the soil surface especially at night and in the morning feeding and can accidentally ingest the eggs of GI tapeworm in the manure. Cysticercoid larval stage develop in the mite and sheep

get infected when they ingest herbage containing mites with infective stage cysticercoid. heavy infection in lambs causes diarrhoea and poor weight gain. (Hansen and Perry., 1994).

**2.3.1.7 Diagnosis of helminthes**

**Worm egg counts (WEC)**

Which is an indicative of the worm burden in lambs, use to monitor nematode infection over time and to assess anthelmintics efficacy. It's rapid, easy and inexpensive to conduct. It's an important component of worm control programme as it monitors the success of worm control programme and it's used to detect seasonal variation in worm burdens (Tailor *et al,* 2007, Kym, 2018).

Strongyle eggs are the first diagnostic stage of nematode infection. Morphologically they are ovoid in shape, thin translucent outer shell with morulla inside (Hansen and Perry, 1994). *Haemonchus contortus, Trichostrongylus* spp and *Oesophagostomum spp* eggs measure 80x45, 80x40, 80x45 micrometers respectively. *Trichuris ovis, Ostertagia* spp and *Nematodirus spathiger* measures 75x35, 80x45 and 200x90 micrometers respectively (Foreit, 2001).

**Fecal culture**: Nematodes eggs are difficult to identify them into genus. Fecal sample are culture at an incubation temperature of $27^0$c for 7-10 days and larvae harvested using Baermann's technique. Baermann's technique is based on active larval migration from suspended faeces in water and their eventual collection and identification (Hansen and Perry, 1994). Identification and differentiation is done based on their anterior and posterior morphological features (Hansen and Perry 1994),

The genera of worms' present can also be identified using quantitative polymerase chain reaction (PCR) which is faster, more accurate and cheaper (Kym, 2018, Coles *et al.*, 2006).

**Total worm counts**: Mature and immature adult worms are counted in abomasum, small and large intestines, this give most definite measure of worm burden. It provides information on the number, genera of worms' present, and proportion of mature and immature worms. However, its expensive,

labour intensive, need sheep to be sacrificed, gross and microscopic examination if gastro-intestinal sections and contents (Kym, 2018).

### 2.3.2 Sheep coccidiosis

Coccidia are protozoan parasites causing damage to the lining of the small intestines affecting nutrient absorption hence weight loss, stunted growth, and diarrhoea containing blood and mucous. Intensive grazing systems, poor sanitation, overcrowding, weather changes, environmental stress, nutrition deficiency, changes in feed, weaning, illness, parasites, pregnancy and lactation increase the risk of coccidiosis (Constable *et al*, 2012). Coccidia of sheep include; *Eimeria parva, Eimeria punctata, Eimeria ahsata, Emeria bakuensis, Eimeria crandallis, Emeria faurei, Eimeria granulosa, Eimeria gonzalezi, Eimeria gilruthi, Eimeria intricata, Eimeria marsica, Eimeria ovinoidalis, Eimeria pallida, Eimeria weybridgensis, Eimeria dalli* (Tafti and Hashemnia, 2017; Kym, 2018; Soulsby, 1982; Foreit, 2001).

#### 2.3.2.1 Life cycle of coccidia

Coccidiosis in sheep is caused by genus Eimeria which cause considerable loss in sheep production. Understanding the life cycle of coccidia help visualize their effects in the host animal. Coccidia are obligate intracellular parasites developing within epithelial cells' cytoplasm causing hyperplasia and cell death. Extend and occurrence of damage is determined by species of *Eimeria* involved, stress, amount of infective dose of oocysts, host factors like level of immunity, age, physical and physiological status). Life cycle of coccidia involves an exogenous phase whereby the oocyst matures through sporogony and parasitic endogenous phase which take place inside the host and characterized by asexual and sexual multiplication (Constable *et al*., 2012).

Unsporulated oocyst is excreted through faeces and 2-7 days later it becomes infective stage (sporulated oocyst) under suitable temperature, adequate moisture and oxygen. The sporulated oocyst

is ovoid with a thick wall hence very resistance to external environment and survive for several months before being ingested by a suitable host. Upon ingestion by a suitable host it undergoes excystation. The initial single cell divides into four sporoblast, each then develops into one sporocyst and two sporozoites develop inside each sporocyst.

Sporozoites infect cells in the intestinal mucosa through penetration and changed into first generation schizont. Schizogony (two asexual multiplications) takes place only in the small intestine or in small followed by large intestines. The resultant schizonts or schizozoites then penetrate the epithelial cells and form motile merozoites.

Second generation schizogony take place in the large intestines resulting in second generation merozoites which invade the epithelial cells and undergoes sexual multiplication (merogony) to form macro and micro gametes which fuse into a zygote and finally form unsporulated oocyst. The oocyst is released to environment through faeces and life cycle starts again (Tafti and Hashemnia, 2017; Soulsby, 1982; Foreit, 1999; 2001).

#### 2.3.2.2 Clinical signs of coccidiosis

Occurs 4-6 months post-infection in lambs and determining factors include; age, physiological status, genetic susceptibility, host immunity, health status, physical condition and stress factors like over-crowding, weather, change of environment, dietary changes, climatic changes, and movement (Constable *et al.*, 2012). Coccidia invade and destroy intestinal cells causing anaemia, loss of electrolytes and disrupt nutrient absorption, thus causing decreased productivity, low growth performance, high mortality and high cost of treatment and prevention (Chartier and Paraud, 2012). Major clinical signs include; diarrhoea at first watery faeces with clumps of mucous, later color changes from brown to yellow or dark tarry due to hemorrhagic diarrhoea, weight loss with marked dehydration, listlessness, tenesmus and pale conjunctiva, inappetance and finally mortality (Maingi

and Munyua, 1994).

### 2.3.3 Prevalence of gastrointestinal parasites.

Prevalence of gastrointestinal (GI) parasites in sheep indicates the positive cases in a given population and its expressed as a percentage of the total. The prevalence is influenced by grazing management, climatic seasons, age, sex, weather condition, physiological status, nutrition, host-parasite interaction, animal husbandry and anthelmintic usage (Belina *et al.,* 2017).

Prevalence of GI parasites infection in sheep has been reported in; Pakistan (94%) Ruhoollah *et al.,* (2021), India (68.64%) Bhowmik *et al.,* (2020), Ghana (98.2%) Owusu *et al.,* (2016), Nigeria (64%) Maimadu *et al.,* (2020). In Kenya prevalence of GI parasites have been reported in; Magadi Division (Maichomo *et al.,* 2004), Nyandarua district (Maingi, 1996), Kajiado district (Ng'ang'a, 2002).

In farms increase in prevalence is mostly attributes to the challenge of AR, pasture contamination and host immunity for example in ewes there is increase egg output around parturition time a phenomenon called periparturient rise (Ng'ang'a *et al.,* 2006). Prevalence of coccidia in sheep is affected by management factor, climate, weather condition, breed, stress, level of immunity, physiological and health status. Many studies on prevalence of coccidia has been reported in many parts the world for example; Iran (54.68%) Altaf *et al.,* (2014), Ethiopia (59.6%) Ayan *et al., (*2009), Nyandarua District (43.6%) Maingi and Munyua, (1994) and Kisumu municipality (35%) Kanyari *et al.,* (2009).

The intensity of gastrointestinal parasite infection is given as the mean egg per gram (EPG) or oocyst per gram (OPG) of feces tabulated in all the samples that tested positive to the parasite. Values of fecal egg count for helminthes are categorized as free, EPG <500 is low or mild infection, EPG (500-1000) as medium and EPG >1000 is high or severe infection (Soulsby, 1982). Coccidial infection are categorized based on oocyst per gram (OPG) as free, low or mild (50- 799 OPG), moderate (800-

1200 OPG) and > 1200 (OPG) as high or severe infection (Urquhart *et al*, 1996).

### 2.4 Control of gastrointestinal parasites

It's very difficult to eradicate gastrointestinal parasites but it's easy to control them meaning the parasites burden in the host are suppressed to a level where no economic loss is felt (Ng'ang'a, 2002). The main aim in controlling gastro-intestinal parasites is to break their life cycle which can be achieve through integrated parasite management involving both chemical and non-chemicals methods.

Integrated parasite management refers to a system where combined approaches are used considering economic factors, epidemiology, resistance status, production system and management structures. Strategic treatment using anthelmintics and coccidiostats based on proper epidemiological studies, proper hygiene, pasture and animal management, proper nutrition, selective breeding and vaccination (Waller, 1987; Sundar, 2004; Sissay, 2007).

#### 2.4.1 Control of helminthes

Over the years control of helminthes has been achieved through use of anthelmintics (chemical control method) but due to expanding development and diffusion of anthelmintic resistance, novel solution has been explored for more sustainable control. The solution involves; limit the contact between the hosts and the infective larvae in the field through grazing management and use of biological control agents, improving host response against helminthes infections through genetic breed selection (breeding for resistance) and nutritional manipulation and finally use of non-conventional anthelmintic materials (plant or mineral compounds) which can eliminate worms or negatively affects parasites biology (Sundar, 2004).

#### 2.4.1.1 Control by anthelmintic treatment

Anthelmintics drugs have been used extensively to control helminthes. Treatment may be done in three ways; empirical, curative and preventive. Empirical is anthelmintic treatment without any strategy while curative is delayed treatment until clinical signs or death occurred and preventive treatment reduce excessive contamination on pasture and minimize host susceptibility (Ng'ang'a, 2002).

**Strategic treatment** is based on knowledge of the seasonal changes in infection and the regional epidemiology of the gastrointestinal worms. It's done at a specific time in the year or stage in a management programmes purposely to reduce worm burden and pasture contamination. Strategic treatment involves treating at the onset of worm season and during favorable condition for development of free-living stages on pastures, this lowers accumulation of worms and risk to susceptible animals. Strategic treatment protects young animals at weaning as they suffer nutritional stress and eliminate pasture contamination in adults. Strategic treatment in breeding ewes reverse Periparturient weakened immunity and it is preferably done a month before and after parturition (Ng'ang'a, 2002).

**Tactical treatment** is done when expecting development of parasitic infection especially during rainy seasons, poor nutrition and when a naive animal is introduced to contaminated pastures (Ng'ang'a, 2002). Developing countries have poor plan of prophylactic control of gastrointestinal helminthes since anthelmintics are extensively utilized. Inadequate knowledge of parasites epidemiology results in either suppressive treatment at close intervals and at the end of the pre-patent period or treating whenever clinical signs appear. Suppressive treatment effectively reduces population of parasites and production losses for short term. However, it leads to high selection of resistant parasites. Curative approach on the other hand results in high risk of production losses at uncontrollable rate and

occurrence of clinical diseases but there is less strong selection of anthelmintic resistance (Kym, 2018).

In Kenya treatment is done when animal show clear signs of helminthosis (Githigia, 2000; Kinoti, 1994). Regular anthelmintic treatment is done monthly to six weeks in wet seasons and it is done throughout the year when dry season does not exceed three months (Githigia, 2000). Visual estimation of animal weight for dose determination is a common practice by livestock farmers (Maingi, 1996), however anthelmintic dosing should be determine based on weight of individual animal or weight of the heaviest animal in the group. Unawareness by farmers on drug administration, dosing and deworming frequency enhance development of anthelmintic resistance (Maingi, 1996; Taylor *et al.*, 2002).

Mostly used classes of broad spectrum anthelmintics are; benzimidazoles (BZ)- albendazole, macrocyclic lactones (ML)- ivermectin, imidazothiazoles/Tetrahydropyrimidines (LV)- levamisole (Sara, 2011). Narrow spectrum anthelmintics are Salicylanide/nitrophenols- morantel and /organophosphates (OP). OPS are very toxic though they are still in the market (Sara, 2011). These are the currently available anthelmintics in the markets and new anthelmintics having different mode of action is not expected in future, hence the need to maintain their efficacy to ensure sustainable productivity, health and welfare of animals and humans (Coles *et al.,* 2006). Availability of these chemicals at affordable prices and limited knowledge makes farmer use them indiscriminately which has potentiated AR (Githigia, 2000).

**Mechanism of action.**

**Benzimidazole include:** albendazole, fenbendazole, thiabendazole. They bind to tubulin leading to disruption of intracellular micro tubule transport system and inhibition of tubulin polymerization and micro tubule formation and eventually damages the parasites' tubulin. They also inhibit and disrupt

metabolic pathways (Abongwa *et al*., 2017; Moreno, 2017).

**Imidazothiazoles/ tetrahydropyrimidines**

Pyrantel and morantel are the anthelmintics found in class and they act as nicotinic receptor agonist in both sympathetic and parasympathetic ganglia of worms. They interfere with carbohydrates metabolism (Abongwa *et al.*, 2017; Moreno, 2017).

**Macrocyclic lactones (**Avermectin, melbemycin, ivermectin)

Causes paralysis and death by enhancing release of GABA at the pre-synaptic neurons which prevent post-synaptic receptor stimulation. It also binds to receptors that increase permeability of membrane to chloride hence inhibiting function of the nerve cell (Abongwa *et al.*, 2017; Moreno, 2017).

**Narrow spectrum anthelmintics**

Include: Salicylanides and nitrophenols (closantel) and organophosphates. Salicylanides and nitrophenols **(**closantel) causes blockage of acetylcholine leading to flaccid paralysis and worm expulsion while organophosphates inhibit cholinesterease in nerve synapse causing spastic paralysis of the parasites (Abongwa *et al.*, 2017, Moreno, 2017.

**2.4.1.2 Pasture management**

Pasture management breaks internal parasites life cycle especially infective larvae which survive on pasture for long duration. Infective $L_3$ larvae of *Haemonchus* species can survive on pasture for extended periods of time. Sheep are more susceptible to parasitic infection, unlike other animal they graze much closer to the ground and can graze in areas highly contaminated with faeces. These increase sheep exposure to higher numbers of larvae that they can ingest.

Pasture management allow enough time for pastures to rest thus lowering the number of infective larvae and allow new pasture growth. To avoid pasture related parasite problem is to avoid overgrazing areas of pasture. Pasture management involves mixed grazing, alternate grazing and

rotational grazing practices (Githigia, 2000; Ng'ang'a, 2002). Good rotational grazing in combination with an efficient anthelmintic program should significantly reduce parasites in the flock.

#### 2.4.1.3 Breeding for genetic resistance

Helminthes control can be achieved by breeding susceptible sheep breeds with genetically resistance breeds for example merino breed have been identified to be resistance to *Haemonchus contortus* (Ng'ang'a, 2002).

#### 2.4.1.4 Vaccination

It's another way of controlling helminthes though helminthes vaccines have been proven difficult to develop. Treatment and control of haemonchosis has become a great challenge in sheep production which has led to development of a vaccine called Barbervex. It is an adjuvant vaccine which has natural H-11, H-gal-GP antigens obtained from adult *Haemonchus contortus*. This vaccine has been approved in Australia with proven efficacy in sheep and goats (Vanhoy *et el*.,2018). Another nematode parasitic vaccine that has been successfully developed is the radiation- attenuated vaccine for lung worm *Dictyocaulus vivipara* (Githigia, 2000, Ng'ang'a, 2002).

#### 2.4.1.5 Biological control

Biological control reduces the number of infective larvae reaching the animal and boost development of natural immunity. Biological control agents include bacteria, virus, protozoa and fungi. Nematode destroying fungi *Duddingtonia flagran* has receive a lot of attention and fungal materials should be in dung for easy entrapment and killing of nematodes' larvae (Githigia, 2000, Ng'ang'a, 2002).

### 2.4.2 Control of Coccidiosis

Treatment is achieved using coccidiostats but intensive use can lead to development of resistance, thus prevention is basic and it relies on improved hygienic condition, adequate nutrition and reduction of stressors (Constable *et al.*, 2012; Wrights and Coop, 2007). Sulphonamides, amprolium and

monensin are the mostly used coccidiostat. Decoquinate, toltrazuril and diclazuril acts on the entire cycle of coccidia and have both curative and preventive effects (Constable *et al.*, 2012).

Effective GI parasite control involves; treatment with anthelmintic and coccidiostats, hygienic management, good nutrition, grazing management and breeding for resistance (Baker *et al.*, 2002), this will minimize production losses and maximize sheep's immunity to these parasites as well as reducing the chances of development of drug resistance as well as enhancing sustainability of animal production (Githigia, 2000). For profitable sheep production system sheep must develop a strong immunity against gastro-intestinal parasites and it increases with age and exposure to infection (Kym, 2018).

### 2.4.3 Host immunity

Immunity reduces worm burden and once it develops a stable number of worms resides in the gut and ingestion of infective larvae are rejected or their development is arrested. Exposure of parasites to young sheep is necessary for development of immunity. For effective control of *Trichostrongylus* spp. and *Haemonchus contortus*, timing must be done so that anthelmintics administration coincides with development of immunity and these results in re-establishment of a reduced worm burden (Kym, 2018). Host immunity to helminthes is influenced by age, sex, nutrition, genetics, physiological status (lactation and pregnancy (Ng'ang'a, 2002).

#### 2.4.3.1 The manifestation of immune competence includes:

Host immune competence manifest in the following way; failure of development of infective larvae to adult worm in the gut, inhibition of larval stage four to develop to adult worm, reduction in the fecundity and length of adult female and expulsion of adult worms from the gut (Ng'ang'a, 2002). Self-cure for *Haemonchus contortus* can occur through rapid re-infection (vaccination) and improved nutrition (intake of high-quality pasture) and it's manifested by sudden egg count reduction to low

counts or zero from high levels (Ng'ang'a *et al.,* 2004). Self-cure commonly occurs at the end of rainy season when infective larval intake stimulates hypersensitivity reaction in abomasum and intestines evacuating heavy adult parasite load ((Ng'ang'a, 2002). In Kenya this phenomenon has been reported to occur in both dry and wet season (Ng'ang'a, 2002; Ng'ang'a *et al,* 2004; Gatongi *et al,* 1998).

**2.5 Anthelmintic resistance (AR) occurrence and its detection/testing.**

**2.5.1 Anthelmintic resistance occurrence**

Anthelmintic resistance (AR) occurs when parasites are able to withstand amounts of anthelmintics that would otherwise eliminate helminthes belonging to similar stage and species. It's genetically transmitted in worms that survive the treatment (Geary *et al.,* 2012). Anthelmintic resistance (AR) is the greatest threat to grazing livestock production and mainly small ruminant industry globally and its attributed to frequent use of anthelmintics (Mphahlele *et al*., 2019). AR has been reported in USA (Torres *et al*., 2012), Australia (Besier and Love 2003). In Africa anthelmintic resistance has been reported in South Africa (Tsotetsi *et al*., 2013), Zimbabwe (Matiko *et al*., 2003), Zambia (Gabriel *et a*l., 2001) and Kenya (Maingi, 1991; Mwamachi *et al*., 1995; Maingi, 1996; Gakuya *et al*., 2007; Ng'ang'a *et al.,* 2010).

Side resistance occur when helminthes become resistant to drugs of the same class of anthelmintic, whereas resistant against two or multiple drugs of different classes of anthelmintic is called cross or multi drug resistance (Torres *et al.,* 2012). Studies conducted in Kenya indicated that Abomasal worm *Haemonchus contortus* have develop resistance to benzimidazole, (Maingi, 1991), ivermectin and closantel (Mwamachi *et al.,* 1995) and benzimidazole, levamisole and ivermectin (Waruiru *et al.*, 1997; Ng'ang'a *et al.,* 2005).

**2.5.2 Anthelmintic resistance testing**

The growing significance of AR has led to development of reliable and standardized testing methods.

***In_vivo* test** include; faecal egg count reduction test (FECRT) described by Coles *et al.*, (2006), controlled test described by wood *et al.*, (1995).

***In_vitro* tests**: Taylor, (2002) described an egg hatch assay; larval paralysis and motility assay, while Jackson *et al.*, (1992) described larval development assay; Taylor, (2002) also described tubulin binding assay and larval development assay and Coles *et al*, (2006) described micro-agar development test (MALDT).

**2.5.2.1 *In_vivo* test diagnostic method**

**2.5.2.1.1 Faecal egg count reduction test (FECRT)**

FECRT remain the main method that is used to test resistance with all groups of anthelmintics. Eggs in faeces are counted at the time of anthelmintics administration and at a specified time after treatment based on the anthelmintics used. If more than 25% of helminthes are resistance this test becomes reliable (Coles *et al.*, 2006).

The principle behind this method is based on the ability of the anthelmintics being tested to lower the concentration of egg per gram of faeces (EPG) at day 14 post- treatment by more than 95% as compared with pre-treatment EPG. A minimum of ten animals are required per treatment group as well as in the un-treated control group and the EPG should be more than 150 or 200 before treatment (Coles *et al.*, 2006).

**FECRT protocol for sheep**

**Selection of animals**

Lambs 3-6 months old that have been bred in the farm or adults with more than 150 EPG upon screening are selected. These animals should not have received any anthelmintic treatment in the last 8-12 days. The selected animals are randomly allocated into each treatment groups with at least ten each. The control group should have equal number of animals as the treatment groups and its purpose

to help monitor natural egg count changes within the test period (Coles *et al.*, 2006).

**Anthelmintics treatment**

Anthelmintics are administered base on the manufacturer's instruction, dose in mg/kg BW should be administered with calibrated syringes (Coles *et al.*, 2006).

**Faecal sample collection**

Pre and post treatment faecal samples should be collected directly from the rectum, placed in sealed container and transported to parasitology laboratory for egg count and if the mean egg count of any group is less than 150 EPG before treatment, then the detection of resistance become unreliable (Coles *et al.*, 2006). Post-treatment faecal sample collection for levamisole, albendazole and ivermectin should be done 3-7 days, 8-10 days and 14-17 days later respectively. However, if testing all the groups in one flock of sheep then the recommended post-treatment faecal samples should be collected 10-14 days later (Coles *et al.*, 2006). Faecal sample should be processed as described in the manual of veterinary parasitology.

**Data analysis and interpretation**

arithmetic mean, percentage reduction and 95 percent confidence interval should be calculated. The arithmetic mean is used instead of geometric mean since it is easily calculated and gives a better worm egg output estimate. The formula for percentage reduction is as follows: FECR % = $100(1-Xt/Xc)$ where;

Xt represents the mean egg count of the treated group at 10-14 days

Xc is the mean egg count of the control group at 10–14 days.

Resistance is declared when the percentage egg count reduction is less than 95% and the lower confidence level at 95% is less than 90%. If only one of the two criteria are met, resistance is suspected.

Results for FECRT does not fully guarantee an accurate estimate of anthelmintic efficacy since it only test variation of egg produced by the mature worms before and after treatment as there is no direct correlation between the egg output and number of worms (Coles *et al.,* 2006). An effective anthelmintic should clear the helminthes so that no egg should be found in faeces at the 14 day after administration (Coles *et al.,* 2006).

**Advantages**

FECRT is robust and simple test, does not need highly trained individual, valuable resources, complex tools and can be performed anywhere and applicable for commonly used anthelmintics as well as being used in most animals including goats, sheep, horses, pigs and cattle (Coles *et al.,* 2006).

**Disadvantages:** number of animals needed per group and EPG before treatment is high, low level anthelmintics resistance can't be detected using this method (Coles *et al.,* 2006).

Anthelmintic failure to reduce egg counts effectively means there is resistance, however, in a natural infection only one species is resistance among the many nematodes species, hence larval stage three should be cultured from faecal eggs from both treated and control groups separately (Coles *et al.,* 2006).

**Faecal culture**

Fifty grams of same size faecal samples from each treatment group are put together, finely broken using a spatula. They are supposed to be crumbly and moist not watery. Vermiculite (sterile peat moss or fine charcoal) is added into wet faeces to make them sterile before culture. Glass dishes for culture are filled with the faecal mixture, loosely covered and cultured in an incubator at 22-27$^0$c for 7-10 days. Larvae are either collected using a Baermann's apparatus or by letting the mixture to stand in a petri dish with water, or by suspension of the mixture in water in muslin. Lugol's iodine is used to treat the larvae and identification of 100 larvae should be done. Egg counts can be placed to genera

depending on 100 larvae results and determination of individual efficacies per genus is done (Coles *et al.,* 2006).

#### 2.5.2.1.2 Controlled test

It's used to determine anthelmintics efficacy through comparison of parasites populations in non-treated and treated groups of animals. Worm burden of artificially infected animals are compared with that of susceptible animals or nematodes isolates of resistant suspects.

Animals with parasites infestation are separated randomly into treated and un-treated groups and post-mortem recovery, counting and identification of parasites is done at a desired interval after medication (10-15days). This test is only used to confirm anthelmintic resistance at species level and evaluate the effects of larval stage three (woods *et al* 1995; Coles *et al.,* 2006).

### 2.5.3.1 *In_vitro* diagnostic methods

Many invitro tests are available which can be utilize to determine resistance level. However, they need trained personnel and laboratory tools. Infections that are mono-specific are used when carrying out these tests as it's not easy to interpret field infection results which have more than one parasite species. For comparison to be achieved drug resistant and drug susceptible standard laboratory strains should be maintained.

**Table 2.1: *In_vitro* AR diagnostic tests and respective anthelmintics they test (Coles *et al.*, 2006)**

| ***In_vitro*-tests** | **Anthelmintic tested** |
|---|---|
| Egg hatch assay | Levamisole – morantel |
| | Albendazole |
| Larval paralysis and motility assay | Levamisole – morantel |
| Larval development assay | All drugs |
| Tubulin binding assay | Benzimidazole |
| Adult development assay | Benzimidazole |
| Micro-agar development test (MALDT) | Levamisole |
| | Albendazole |

#### 2.5.3.1.1 Egg hatch Assay

Principle: Failure of eggs to hatch in drug in solutions of increasing concentration of drug relative to the control wells (Taylor, 2002). It shows the differences in susceptibility to levamisole and benzimidazole by git nematodes strains. The determining factor is the failure of eggs to hatch as the drug concentration is increased (Taylor, 2002).

**Advantages**

It's an accurate test to assess mixed populations of nematode susceptibility compared to FECRT its economical and can be done more rapidly (Taylor, 2002).

**Disadvantages**

Fresh eggs must be used within three hours after they are voided from the host, because once the process of embryonation starts sensitivity to benzimidazole goes down. It's only applicable on nematodes parasites whose eggs hatch rapidly. The results are difficult to interpret hence not widely used (Taylor, 2002).

#### 2.5.3.1.2 Larval paralysis and motility Assay

**Principle;** ability to differentiate between susceptible and resistant parasites strains, through estimation of the proportion of larval stage three in tonic paralysis upon being incubated with given concentration levels of morantel and levamisole (Taylor, 2002).

**Advantages**

It's a simple and easily carried out, infective larvae stock can be obtained readily, its reproducibility is good.

**Disadvantages**

Early and late addition of anthelmintics to egg suspension complicates the interpretation of results

because the eggs have not developed fully or it's too late (Taylor, 2002).

**2.5.3.1.3 Larval development assay-**

The LDA is used to detect sheep nematodes resistance to the broad spectrum anthelmintics (macrocyclic lactones, benzimidazoles and levamisole).

**Principle**: The principle behind this test involves isolation of nematode eggs from a faecal sample, placing them in wells of a Microtitre plate to develop to infective larval stage three in presence of a range of anthelmintics concentrations. The amount of anthelmintics inhibiting larval development is the same as the concentration that effectively kill helminthes in life animal (Jackson *et al.*, 1992).

**Advantages**

It fast and less manpower needed, as it allows detection of resistance to all the three-broad spectrum anthelmintics in a single farm visit.

**Disadvantages**

It requires a lot of time for counting to get $LD_{50}$, comparison is only achieved with the presence of susceptible and resistant parasites strains and can only provide an indication of anthelmintic resistance in case of macrocyclic lactones (Jackson *et al.*, 1992).

**2.5.3.1.4 Tubulin binding Assay-**

**Principle:** relies on mechanism of action of drugs and it's based on tubulin being bound by benzimidazole differently. Evidence of benzimidazole resistance is the reduction of its affinity to tubulin. Crude tubulin extracts from egg, larvae or adult parasite are incubated with titrated benzimidazole till it attains equilibrium. Using charcoal, the free drug in suspension not bound to tubulin is removed and the label bound to tubulin is sampled and counted using liquid scintillation

spectrophotometry. Tubulin extracts obtained from resistant parasites have reduced binding unlike those from susceptible parasites (Taylor, 2002).

**Advantages**

Its robust, rapid with high reproducibility and sensitivity

**Disadvantages**

Large larval number is required hence does not fit routine field assay and laboratory equipment are very costly.

**2.5.3.1.5 Adult Development Assay**

It detects nematodes that are resistant to benzimidazoles (Taylor, 2002). Only used for research works. Each of the above methods require large amount of capital to run, unreliable, less sensitive and difficult to interpret the results (Coles *et al.*, 2006).

**2.5.3.1.6 Micro-agar development test (MALDT)**

**Principle**: the principle is based on the development of eggs to stage three larvae (Coles *et al.*, 2006).

**Advantages**

Easy to use and reliable, larval stage three can be identified to species levels after the test this gives the type of helminthes present and those surviving (Coles *et al.*, 2006).

**Disadvantages**

It is less important than egg hatch assay and only reliable for levamisole and albendazole resistance testing. Different concentration of fungicide (amphotericin b) and yeast extracts are needed for both equine and ovine nematodes (Coles *et al.*, 2006).

**2.5.4.1 Molecular based test**

The molecular mechanism conferring resistance to benzimidazole in *Trichostrongylus* in sheep and goats involves mutation of phenylalanine to tyrosine at residue 200 of b-tubulin gene isotype 1. The

same mutation can occur at codon 167 in benzimidazole resistance in nematodes (Coles *et al.*, 2006). It can be used as routine test if larval DNA samples that are pooled are used for diagnosis since testing one at time is extremely expensive (Coles *et al.*, 2006).

### 2.6 Control of Anthelmintics Resistance

Anthelmintic resistance can be limited through use of non-chemical control methods, which include: parasite vaccine development, use of animal genotypes that are resistant to parasite infections; understanding the epidemiology of parasites (Ng'ang'a, 2002; Sissay, 2007); nutritional management (Bricarello *et al.*, 2005); biological control (Githigia, 2000); and integrated pasture management enhance control of AR. Rational use of anthelmintics helps preserve the life of available drugs (Waruiru *et al.*, 1998)**.**

## 3.0 MATERIALS AND METHOD

### 3.1 Study area

This study was conducted in Kasarani Sub-County, Nairobi County. Nairobi County is situated at $1^0$ 09'S$36^0$ 39'E and $1^0$ 27'S $37^0$ 06'E, occupying 696 $km^2$, with a sub-tropical climate, temperature of $24^0$ C while Kasarani Sub-County is located at $01^0$13'44'S $36^0$54'16'E. Kasarani Sub-County was chosen due to availability and adequate number of sheep farms and sheep population as per the data obtained from the Sub-County Veterinary Office. Gastrointestinal parasites have limit sheep farming in the area with complains of anthelmintic therapy ineffectiveness by farmers. There have been suspect cases of anthelmintic resistance by veterinary service providers in Kasarani. Its proximity to the Parasitology Laboratory, at the University of Nairobi made it easy for immediate processing of faecal samples and reduced cost of travelling.

### 3.2 Study design

A cross-sectional study was conducted June 2021 to determine the prevalence of gastro-intestinal parasites in 30 selected sheep farms. The owners of these farms were interviewed using semi-structured questionnaires to assess knowledge, practice and attitude towards control and treatment of helminthes. An experimental design was also conducted to test for anthelmintic resistance, whereby sheep positive for gastrointestinal nematodes (EPG > 200) were randomly placed into three equal treatment groups and one control group.

### 3.3 Farm selection

Based on the information from Department of Livestock and Fisheries both in Nairobi county and Kasarani Sub-County offices, 30 farms keeping at least 20 sheep were selected for prevalence testing this ensured that the sample size was representative of the population. Ten farms with a minimum of 40 sheep testing positive for strongyle were further selected for anthelmintic resistance testing as per

the protocol for fecal egg count reduction test (FECRT) (Coles *et al.*, 2006) which states that a minimum of ten sheep is required per treatment group.

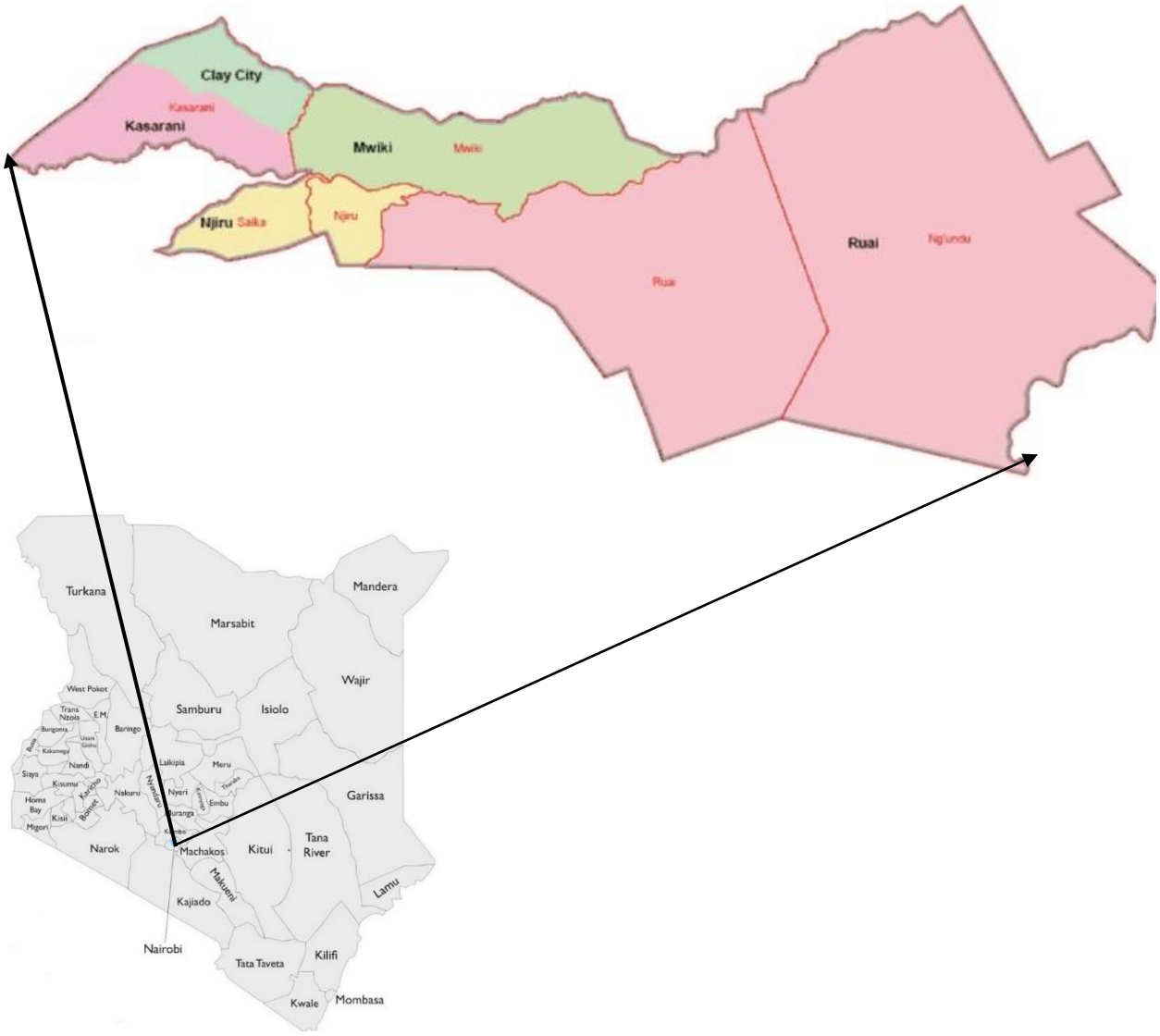



**Figure 3.1: A Sketch of map of Kenya showing location of Kasarani Sub-County in Nairobi County. Source; (Google Map).**

### 3.4 Study animals

Mixed breeds of sheep of all ages, both male and female, on the selected 30 sheep farms were used for this study, however, suckling lambs were excluded. For determination of prevalence a large sample size of 1642 was used. All sheep utilized in this study were prospectively approved and a

formal ethical approval document was granted by animal welfare committee of The University of Nairobi Faculty of Veterinary Medicine.

### 3.5 Data collection

#### 3.5.1 Objective 1: Prevalence of gastrointestinal parasites

##### 3.5.1.1 Collection of faecal samples

Using gloved hands, faecal samples were collected from the rectum of study sheep, placed into labelled faecal pots and transported in cool boxes to Parasitology Laboratory, Department of Veterinary Pathology Microbiology and Parasitology, University of Nairobi, for processing and analysis. In the Laboratory, the faecal samples were refrigerated at $4^0$C for later analysis.

**Parasitological examination**: In the Laboratory, faecal floatation, sedimentation, modified McMaster techniques and faecal culture were performed as described (MAFF, 1986).

##### 3.5.1.2 Modified McMaster technique

McMaster technique uses a counting chamber to examine a known volume of faecal suspension microscopically, therefore, it was used for counting strongyle and tapeworm eggs and coccidial oocysts in faecal suspension. Twenty-eight (28ml) of sodium chloride floatation fluid with 1.2-1.3 specific gravity, were measured using a measuring cylinder and poured into a beaker and 2 grams of faecal samples were added into the beaker. Using a spatula individual faecal materials was broken to smaller sizes to separate the eggs and oocysts from the faecal debri. Tea strainer was then used to sieve the mixture into a clean beaker. A sample of the mixture was taken using a Pasteur pipette to charge the two chambers of McMaster slide (MAFF 1986).

The charged McMaster slide was left to stand for five minutes before mounting on light microscope stage for egg and oocyst counting. Egg and oocyst per gram (EPG/OPG) of faeces was reported by multiplying the number of eggs by a factor of 50, each egg observed represent 50 eggs per gram of

faeces. From the counts the prevalence of helminthes and coccidial infestation was tabulated by expressing the positive cases as a percentage of the total.

### 3.5.1.3 Sedimentation technique

Sedimentation technique was used to determine trematode eggs by concentrating them in sediments since they are heavier than nematodes eggs. Fecal samples for all sheep from each farm were pooled, mixed in a container and 3g of the mixture measured, placed into a container with 50ml of water and thoroughly mixed. The suspension was filtered through a tea strainer into another container. The filtered mixture was transferred into a test tube and allowed to sediment for 5 minutes. Careful removal of the supernatant was done using a pipette.

The sediments were further re-suspended in 50ml of water and allowed to sediment for 5 minutes, the supernatant was discarded afterwards. A drop of methylene blue was added to stain the faecal particles deep blue and with the trematode eggs unstained.

A drop of the stained sediments was placed on a microscope slide, covered with a cover slip before examination.

### 3.5.1.4 Faecal Culture

Most strongyle eggs are similar in appearance hence to determine the helminthes spectrum, faecal samples from each farm were pooled to make approximately 50g of faeces and placed in petri-dish, finely broken down using a spatula while adding water to make them crumbly.

Culture containers were filled with faecal mixture loosely leaded and incubated at $27^0$c for 10 days. Nematode larvae were recovered using Baermann's technique as described by Hansen and Perry (1994) and MAFF (1986) Manual. At least 100 larvae were counted and identified based on size, their anterior and posterior morphological characteristics as described by Van Wyk, and Mayhew (2013) and in MAFF (1986) manual.

### 3.5.2 Objective 2: Knowledge, practice and attitude survey

Seventy-seven respondents (30 farmers, 23 agricultural and veterinary drug stores sales persons and 24 veterinary service providers) were interviewed using semi-structured questionnaires to assess knowledge practice and attitudes towards treatment and control of gastrointestinal parasites. Most questions were closed ended for precise responses, to minimize variations and ease data analysis. During farm visits observations were made on the type and condition of sheep housing, health status of the sheep, their surrounding environment and pasture cover.

### 3.5.3 Objective 3: Investigation of anthelmintic resistance

#### 3.5.3.1 Farm and animal selection

Upon prevalence testing ten farms with a minimum of 40 sheep positive for strongyle were randomly selected. Animal recruited for anthelmintic resistance testing were selected as described by Coles *et al*., (2006). Therefore, in each farm 40 sheep (minimum of ten sheep in each treatment group and control group) with more than 200 EPG and had not receive any anthelmintic treatment in the last 8-12 days were selected. The selected animals were randomly assigned into three treatment groups (1, 2, 3) and one control group (4) of ten sheep each. The first three groups were treated with albendazole, levamisole and ivermectin respectively while group 4 (control group) didn't receive any anthelmintic treatment.

#### 3.5.3.2 Anthelmintics administration

The classes of drugs used for anthelmintic resistance testing were those known and frequently utilized by farmers in the study area as shown in Table 3.1 below. These anthelmintics were administered as per the manufacturer's recommendations.

**Table 3.1: Classes of anthelmintics drugs, dosage rate and route of administration used for fecal egg count reduction test (FECRT), with their respective treatment groups (animal groups).**

| Family name | Generic name | Trade name and presentation | Animal groups |
|---|---|---|---|
| Benzimidazole | Albendazole | Albafas 10% (100mg albendazole per ml) blue suspension | 1 |
| Imidazothiasole | Levamisole | Levacide (15mg of levamisole hydrochloride per ml) clear yellow solution | 2 |
| Macrocyclic lactones | Ivermectin | Supermec (1% ivermectin) | 3 |

On day 0, the test sheep in the first 3 groups (1, 2. 3) were treated with albendazole (8 mg/kg BW), levamisole (7.5 mg/kg BW) and ivermectin (200 mg BW) respectively. Albendazole and levamisole were administered orally using calibrated syringes, while ivermectin was administered subcutaneously. The control group (group 4) did not receive any anthelmintic treatment.

**3.5.3.3 Faecal sample collection**

On day zero, at least 50g faecal samples (enough for faecal egg count and culture) were collected directly from the rectum of the test animals and individual samples were placed in well labelled faecal pots and caped. The same sampling procedure was followed 14 days after treatment. The samples were transported in cool boxes to Parasitology Laboratory, Department of Veterinary Pathology Microbiology and Parasitology, University of Nairobi and refrigerated at $4^0$C until analyzed.

**3.5.3.4 Faecal sample analysis**

Modified McMaster technique was performed to obtain faecal egg counts (FEC) before and after treatment for the treatment groups and control group. Faecal samples from each treatment group were pooled and cultured at $27^0$ C for 7-10 days, after which larval stage three were harvested and identified. Faecal cultures showed nematodes species resistant to the anthelmintics tested.

**3.6 Statistical analysis of data**

**3.6.1 Prevalence, Intensity and Spectrum**

Descriptive statistics was used to determine the prevalence of GI parasites, by calculating the positivity rate and expressed as a percentage of the total sheep sampled. Using Instat, hypothesis

testing for proportion was done to compare sex and age prevalence of gastrointestinal parasites infection in sheep based on the significance levels (P-values) and the 95% confidence interval for the difference between age and sex prevalence.

Logarithm transformation ($\log_{(x + 10)}$) of the values of eggs per gram (EPG) and oocyst per gram (OPG) of faeces was done to normalize their distribution. Arithmetic mean of eggs per gram (EPG) and oocyst per gram (OPG) of faeces was tabulated to determine the overall intensity of GI parasites infection in sheep at farm level before and after logarithm transformation.

Values of fecal egg count for helminthes were categorized as free, EPG <500 as low or mild infection, EPG (500-1000) as medium and EPG >1000 as high or severe infection (Soulsby, 1982). Coccidial infection are categorized based on oocyst per gram (OPG) as free, low or mild (50- 799 OPG), moderate (800-1200 OPG) and >1200 (OPG) as high or severe infection (Urquhart *et al*, 1996).

Hypothesis for population mean was done to test if there was any significance difference in the intensity of gastrointestinal parasites infection in different age groups and sexes of sheep. Percentage differential counts of larval stage three($L_3$) was done to determine the helminthes spectrum in sheep.

### 3.6.2 Questionnaires survey

Data from the survey questionnaires were entered into Microsoft excel sheet and cross tabulation was done to compare responses and determine the percentage of the respondents.

### 3.6.3 Faecal egg count test (FECRT) data analysis and interpretation

Post- treatment egg per gram of faeces (EPG) were entered into Microsoft excel sheet. Post-treatment arithmetic means for treatment groups and control group was determine and from these means faecal egg count reduction percentages (FECR%) and the lower 95% confidence levels was tabulated for each anthelmintic drug. Anthelmintic resistance was declared presence or absence following the world association for the advancement of veterinary parasitology (WAAVP) guidelines (Coles *et al*,

2006); as follows:

Percentage faecal egg count reduction FECR) =100(1-Xt/Xc), Xt represents mean egg count of the treated group at 10-14 days and xc represents mean egg count of the control group 10-14 days later; 95% confidence interval for the resistance was calculated using excel program available at sydney.edu.au/vetscience/sheepwormcontrol/software/fecr4.xls and verified by manual calculation as described by Coles *et al*., (1992):

Number in group: ($N=\Sigma nj$)

Arithmetic mean counts $Xi=\Sigma iXij/ni$

Variance of counts $S^2i = (\Sigma jX^2ij$ ---$(\Sigma jXij)2/ni)/(ni-1)$

Percent reduction $R=100(1-Xt/Xc)$

Variance of reduction (on log scale

$v= [(s2\ t\ /(ntX^2t)] + [(S^2\ c\ /(ncX^2c)])$

Approximate 95 % confidence interval for R 100 =

$[(1-(Xt/Xc))\ exp\ (\pm\ 2.1\sqrt{v})]$

Upper confidence limit $100[1-(Xt/Xc)\ exp(-2.1\sqrt{v})]$

Lower confidence limit $100[1-(Xt/Xc)\ exp(+2.1\sqrt{v})]$

Where

I denote either the treated (t) or control (c) groups

j denotes each sheep in the group

$S^2i$ denotes the variance on the arithmetic scale, calculated as above or:

$S^2i=\Sigma J\ (XIJ - xi)2/(ni-1)$

According to World Association for the Advancement of Veterinary Parasitology (WAAVP) guidelines anthelmintic resistance was declared if the faecal egg count reduction percentage

(FECR%) was below 95% and the lower 95% confidence limit was below 90% and if one of the above criteria was met anthelmintic resistance (AR) was suspected (Coles *et al*, 2006).

## 4.0 RESULTS

### 4.1 Prevalence of GI parasites in sheep in Kasarani Sub-County, August 2021.

One thousand six hundred and forty-two (1642) sheep were sampled of which, 1209 (73.63%) were adults (above 12 months of age) and 433 (26.37%) were lambs <1year. They were further categorized by sex where 1130 (68.82%) were females and 512 (31.18%) were males.

The overall prevalence of GI parasites infection in 1642 faecal samples collected is shown in Figure 4.1. One thousand six hundred and thirty-six (99.63%) faecal samples were positive for gastro-intestinal parasites out of which 72% were positive for strongyle eggs, 49% for coccidial oocysts and 7% for tapeworm eggs. Sheep fluke eggs were not detected in any of the samples in this area. At farm level the prevalence of strongyle eggs ranged from 31% to 98%, while that of coccidial oocysts and tapeworm eggs recorded highest prevalence of 86% and 38% respectively.

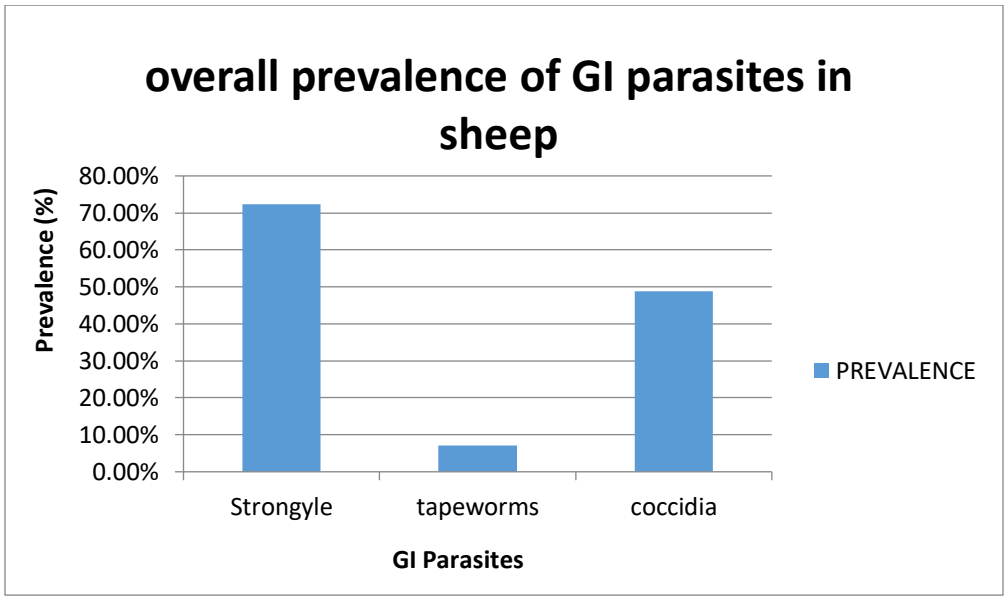



**Figure 4.1: Overall prevalence of GI parasite infection in sheep in Kasarani Sub-County, August 2021**.

### 4.1.3 Prevalence of GI parasites by age group and sex in Kasarani Sub-County, August 2021.

The prevalence of strongyle and coccidial infection tended to be higher in lambs than in adults and higher in females than males though not statistically significant (P values >0.05 (0.7, 0.2 and 0.4, 0.3 respectively). However, the prevalence of tapeworm infection was significantly higher in lambs than adults and in males than females with P values (<0.05) 0.0000, 0.0001 respectively as shown in Table 4.1.

**Table 4.1: Prevalence of GI parasites infection in different age groups and sexes in Kasarani Sub-County, August 2021.**

| Parasites | Sheep category | Number examined | Number positive | Prevalence (%) | 95% confidence level | Z-value | P- value |
|---|---|---|---|---|---|---|---|
| Strongyle | Lambs | 433 | 316 | 73% | -0.039 to 0.059 | 0.41 | 0.6829 |
| | Adults | 1209 | 870 | 72% | | | |
| | Males | 512 | 364 | 71% | -0.028 to 0.066 | 0.80 | 0.4248 |
| | Females | 1130 | 825 | 73% | | | |
| Coccidia | Lambs | 433 | 225 | 52% | -0.015 to 0.095 | 1.43 | 0.1539 |
| | Adults | 1209 | 580 | 48% | | | |
| | Males | 512 | 261 | 51% | -0.022 to 0.082 | 1.13 | 0.2580 |
| | Females | 1130 | 542 | 48% | | | |
| Tapeworm | Lambs | 433 | 61 | 14% | 0.067 to 0.136 | 5.74 | 0.0000 |
| | Adults | 1209 | 48 | 4% | | | |
| | Males | 512 | 56 | 11% | 0.029 to 0.089 | 3.86 | 0.0001 |
| | Females | 1130 | 57 | 5% | | | |

### 4.2 Intensity of Gastrointestinal parasites in sheep in Kasarani Sub-County.

The overall intensity of strongyle infection was 480 mean egg counts (MEC) and coccidial infection was 330 mean oocyst counts (MOC) infection in sheep were low. At farm level the intensity of strongyle infection ranged from mild (32 MEC) to severe (1561 MEC) infection and coccidial infection too (7-2034 MOC).

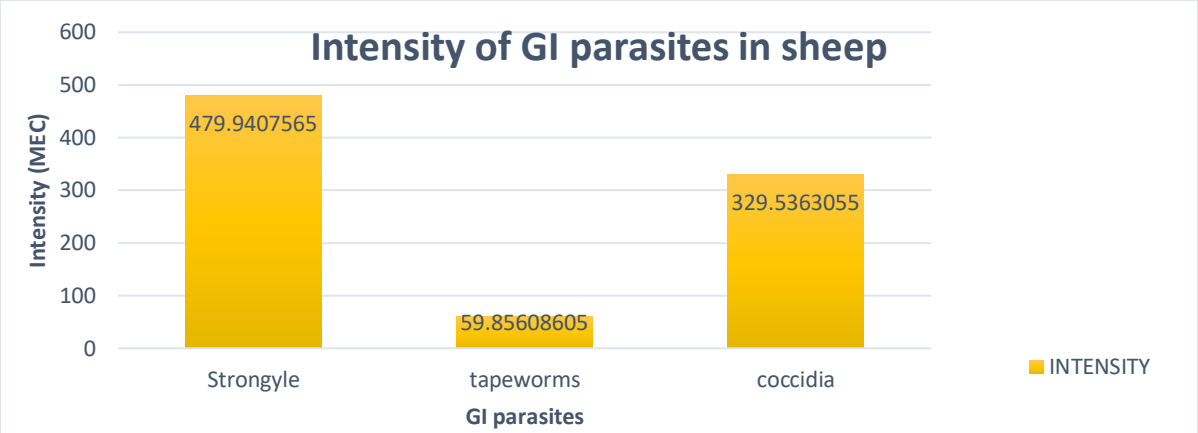



**Figure 4.2: Overall intensity of GI parasites infection in sheep in Kasarani Sub-County, August 2021.**

### 4.2.1 Intensity of GI parasites infection in different ages and sexes of sheep.

The intensity of strongyle (488), tapeworm (94) and coccidial (416) infection tended to be higher in males than females (485, 45 and 306) respectively, though there were no statistical difference between the means. The intensity of strongyle infection was higher in adults (483) than in lambs (472), while the intensity of tapeworm and coccidial infection was higher in lambs than adults, but statistically the difference was insignificant.

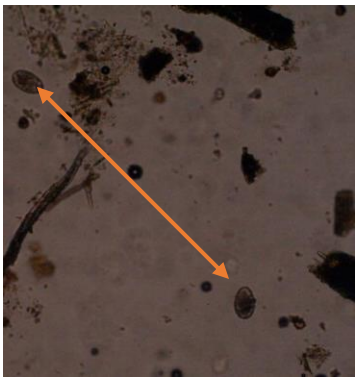
**Table 4.2: Intensity of GI parasites infection in different ages and sexes of sheep in Kasarani Sub-County, August 2021.**

| Parasite group | Sheep category | Number examined | Intensity (log transformed arithmetic means) | 95% confidence level | t- value | P- value |
|---|---|---|---|---|---|---|
| Strongyle | Lambs | 433 | 3.1 | -3.2175 to 0.13294 | -1.81 | 0.0712 |
| | Adults | 1209 | 1.9 | | | |
| | Males | 512 | 1.9 | -3.1679 to 0.18158 | -1.75 | 0.0805 |
| | Females | 1130 | 3.1 | | | |
| Coccidia | Lambs | 433 | 2.5 | -2.0694 to 0.17472 | -1.66 | 0.0979 |
| | Adults | 1209 | 1.3 | | | |
| | Males | 512 | 1.1 | -2.0199 to 0.22354 | -1.57 | 0.1165 |
| | Females | 1130 | 2.6 | | | |
| Tapeworm | Lambs | 433 | 2.1 | 0-1.9623 to 1.1926 | -0.48 | 0.6324 |
| | Adults | 1209 | 0.4 | | | |
| | Males | 512 | 1.5 | -1.9127 to 1.2413 | -0.42 | 0.6764 |
| | Females | 1130 | 1.7 | | | |

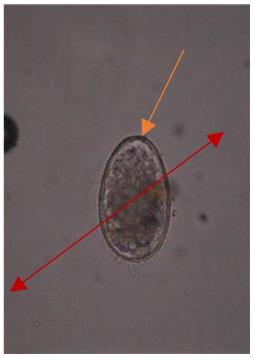
### 4.3 Helminthes spectrum

#### 4.3.1 Parasites Observed

The faecal samples from sheep in different farms were positive for strongyle eggs, tapeworm eggs and coccidial oocysts as shown in Figures 4.3, 4.4 and 4.5 respectively. A typical strongyle egg is approximately 80 micrometer long, broad elliptical in shape with barrel-shaped side walls, thin shelled and has grape-like blastomeres. Strongyle eggs are similar in appearance hence not possible to identify them to genus level. Fecal samples must be cultured to provide larval stage for identification. Tapeworm egg is thick-shelled, irregular shape either triangular or qua-triangular and embryonated with pyriform apparatus. Coccidial oocysts are small in size with a thick shell.

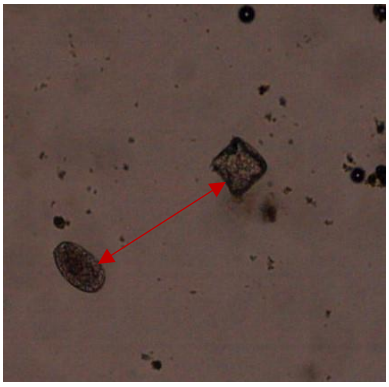


(a)

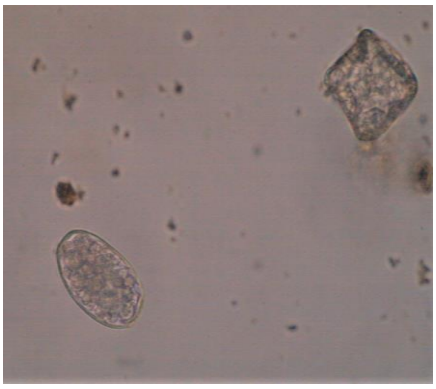


(b)

**Figure 4. 3 (a) and (b): Strongyle egg at x10 at x 40 magnification**

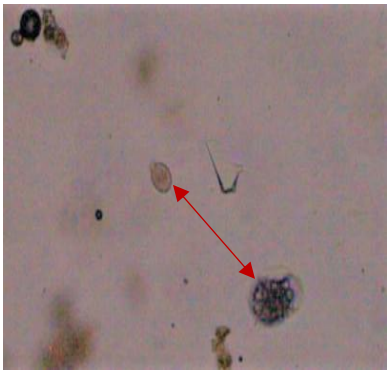


(a).

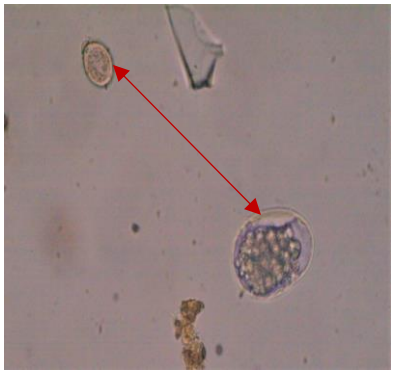


(b).

**Figure 4.4 (a) and (b): Tapeworm and strongyle eggs at x10 and at x40 magnification**



(a).



(b).

**Figure 4. 5 (a) and (b): Coccidial oocyst and strongyle egg at x10 and at x40 magnification**

### 4.3.2 Larval stage three identified.

Larval stage three ($L_3$) were harvested from faecal cultures, differential counting was done and were identified based on their posterior and anterior features as well as their tail sheath measurement. Three helminthes genera were identified namely: *Haemonchus contortus* (90%), *Trichostrongylus* species (5%) and *Oesophagostomum* species (5%) as shown in Figure 4.6. Helminthes spectrum at farm level was mainly dominated by *Haemonchus* species as shown in Figure 4.7.

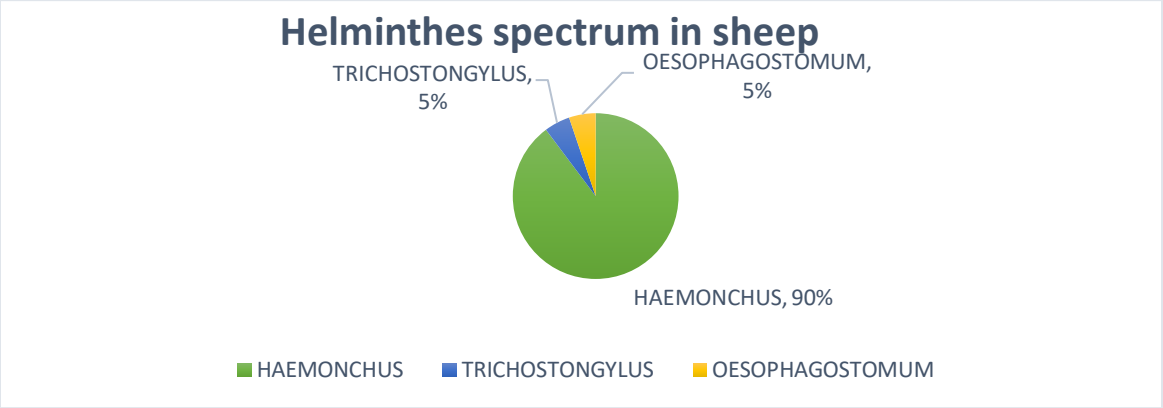



**Figure 4. 6: Overall helminth spectrum in sheep in Kasarani Sub-County, August 2021.**

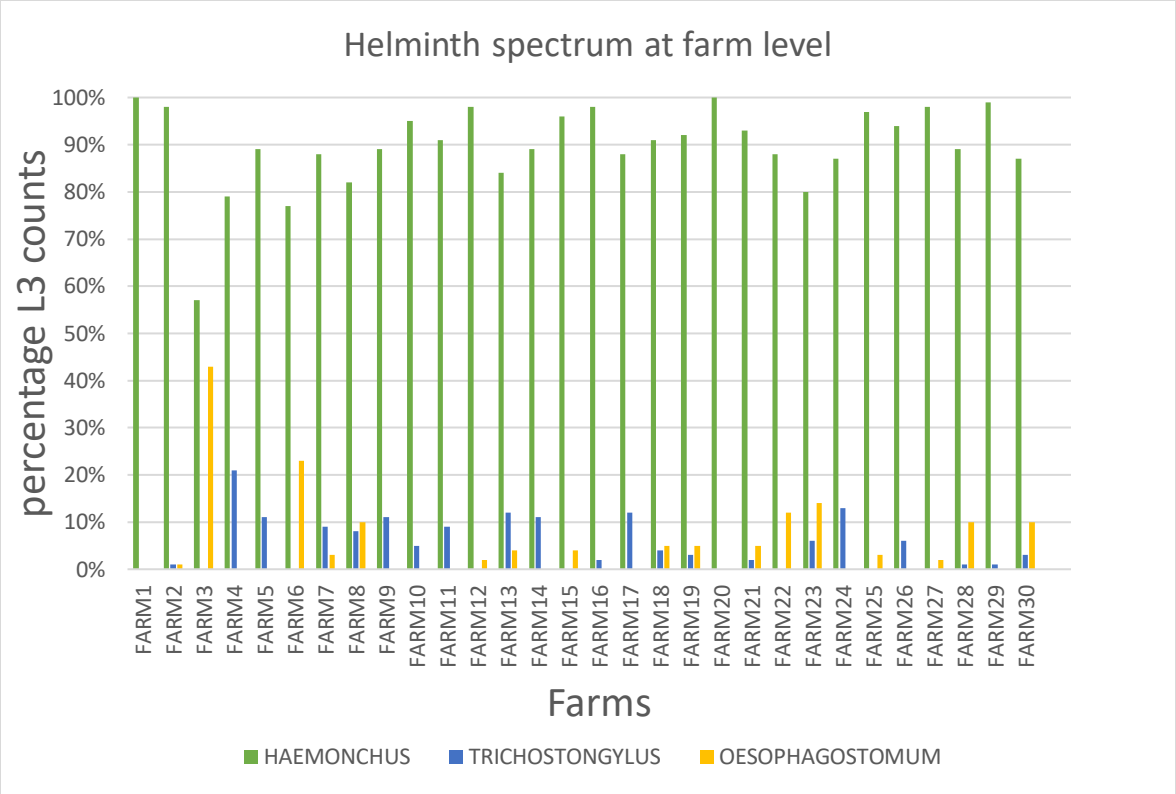



**Figure 4.7: Helminthes spectrum at farm level in Kasarani Sub-County, August 2021.**

The $L_3$ measurement showed that the mean length of *Haemonchus contortus* was 620 micrometers in length (range 500-700 micrometers). They had pointed tail end, kinked tail sheath (Figure 4.8 b) measuring 60 micrometers (range 30-60 micrometers) and bullet-shaped (narrow-rounded) head (Figure 4.8 a). The mean length of *Oesophagostomum* $L_3$ was 900 micrometers (range 900-1200 micrometers). They had broad and rounded head (Figure 4.9 a) and a filamentous tail sheath (Figure 4.9 b) measuring150 micrometer (range 60-180 micrometers). *Trichostrongylus* $L_3$ was short-straight larvae with a mean length of 700 micrometers (range 650-900 micrometers). They had rounded tapered head (Figure 4.10 a) and non-filamentous conical tail sheath (Figure 4.10 b) measuring 30 micrometers (range 20-40 micrometers).

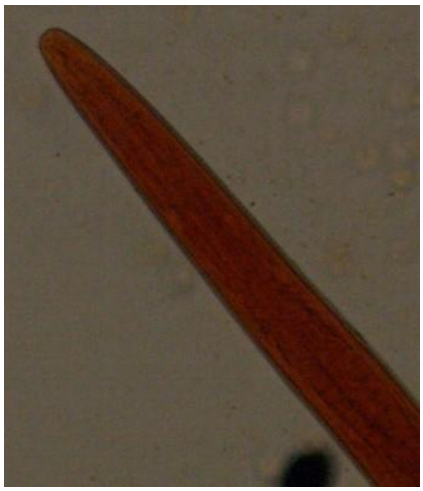
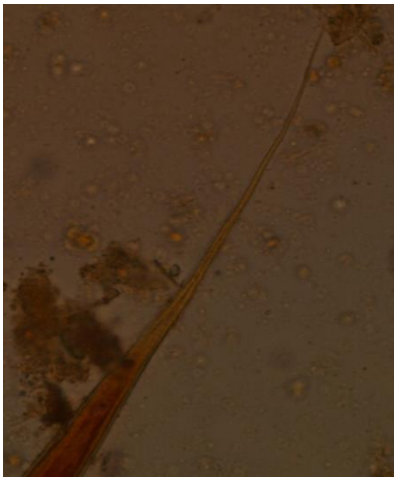
 (a)  (b)

**Figure 4. 8 (a) and (b): Anterior and posterior end of *Haemonchus contortus***

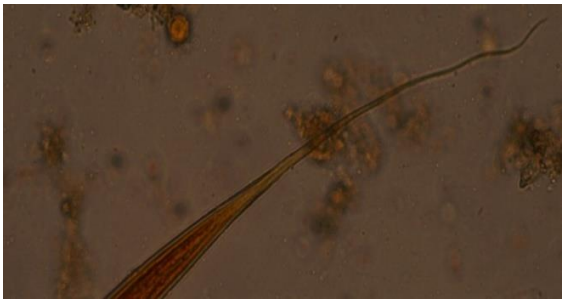
 (a)  (b)

**Figure 4. 9 (a) and (b): Anterior and posterior ends of O*esophagostomum***

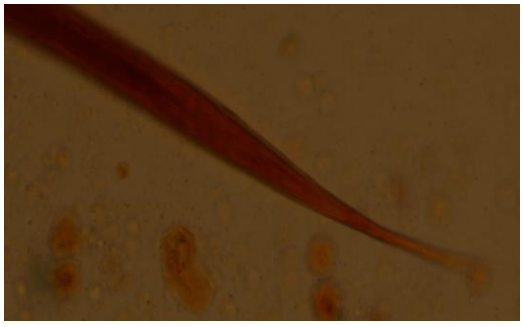
 (a)  (b)

**Figure 4. 10 (a) and (b): Anterior and posterior ends of *Trichostrongylus.***

### 4.4 Knowledge, practice and attitude survey-questionnaire results

Information on knowledge, practice and attitude towards treatment and control of helminthes in sheep was collected using a semi-structured questionnaire administered in personal interviews.

Thirty (30) farmers, twenty-three (23) agricultural and veterinary drugs stores sales person and twenty-four (24) veterinary service providers were interviewed as shown in Table 4.3.

**Table 4.3: Categories of respondents on Knowledge, Practice and Attitude (KPA) towards diagnosis, treatment and control of helminthes in sheep in Kasarani Sub-County, August 2021**

| Gender | **Farmers** | **Veterinary service providers** | **Veterinary Drugs salespersons** | **Total** |
|---|---|---|---|---|
| Male | 27 (90%) | 15(63%) | 9 (39%) | 51(66%) |
| Female | 3 (10%) | 9 (38%) | 14 (61%) | 26 (34%) |
| **Total** | **30** | **24** | **23** | **77** |

**Farmers' Knowledge, Practice and Attitude (KPA) towards diagnosis, treatment and control of helminthes infection in sheep in Kasarani Sub-County**.

Open (3.3%), mixed (26.7%) and communal (70%) farming were practiced in the area. Most of the farmers (80%) constructed temporary pens just outside their homestead where the sheep spent the night since they were released during the day to grazing fields. These pens were full of dusty manure predisposing the sheep to respiratory conditions (coughing and sneezing). Farmers (17%) supplemented their sheep while 83% only depended entirely on pasture especially in farms with larger flock size and where communal farming was practiced.

The anthelmintics available in the study area were albendazole, levamisole and ivermectin belonging to the three classes; benzimidazole, imidazothiazole and macrocyclic lactones respectively. The proportions of farmers using these anthelmintics were 43%, 30% and 27% respectively. Albendazole and levamisole were administered towards the end of rainy season and during dry season respectively, while ivermectin was used for nasal bots, respiratory distress and mange treatment.

Most farmers (90%) purchase these anthelmintics drugs from near-by veterinary drugs stores while other farmers (10%) obtained them from veterinary suppliers. To determine whether a sheep was infected, most farmers (70%) relied on clinical signs such as weight loss, diarrhoea, coughing and

sneezing prior to anthelmintic administration. The ease of administration and price greatly influenced the choice of anthelmintics to use by 70% and 30% of the farmers respectively. Farmers (77%) preferred administering anthelmintics by themselves rather than consulting local veterinary service providers on choice of anthelmintic, dosage and administration.

Majority of the farmers (87%) relied mostly on information provided by drugs sales persons and didn't consult veterinary experts for advice on the anthelmintic usage. Sixty seven percent (67%) of the farmers dewormed their sheep every three months while 33% administered whenever there was sign of helminthes infection. The dosing rate was determined through visual estimation of the sheep's weight (67%) and weighing of individual sheep (33%) as prescribed. Seventy three percent (73%) of the farmers switched to a different class of anthelmintic during every subsequent treatment while 20% alternated after every six months and 7% every year.

**KPA of agricultural and veterinary drug sales persons in Kasarani Sub-County.**

All (100%) the agricultural and veterinary drug stores, stocked the three main classes of anthelmintics for sale. These anthelmintics were sourced from veterinary drugs suppliers (wholesalers) and stored at room temperature. The most frequently purchased anthelmintic from these stores were albendazole (57%), followed by levamisole (26%) and least purchased was ivermectin (17%). Approximately 50% of veterinary drugs sales person were aware that helminthosis was a major cause of poor performance in sheep. Anthelmintic ineffectiveness was as a result drug overuse (78%), improper dosing (13%) and type of anthelmintic used (9%). They advised farmers to consult experts and alternate anthelmintics to avoid development of AR.

**KPA responses by veterinary service providers in Kasarani Sub-County, August 2021.**

Majority of veterinary service providers (75%) confirmed that helminthosis was a major challenge in sheep production in the study area and there was need for a policy to control availability and distribution of anthelmintics.

All veterinary service providers (100%) agreed on the need to alternate anthelmintics classes every year (33% every 2-3 years (17%) or twice a year (50%). During routine veterinary practice 67% of the veterinary service providers had encountered the challenge of AR. The suggested solution to AR by service providers included; strategic deworming, anthelmintic rotation and educating farmers on choice of anthelmintic, dosage determination and administration.

**4.5. Anthelmintic Resistance**

The results of the fecal egg count reduction percentage (FECR%) and their corresponding 95% Cl are shown in Table 4.4. Resistance to all the three anthelmintics (albendazole, levamisole and ivermectin) was detected in one farm. Resistance to albendazole and levamisole was detected in one farm while to albendazole and ivermectin was also detected in two farms. More farms (6) showed resistance to albendazole than to levamisole (4) and ivermectin (4). Suspected (only one criterion either FECR% <95% or 95% CL<90% is met) resistance to levamisole (6) and ivermectin (6) was detected in more farms than to albendazole (4) as summarized in Figure 4.11.

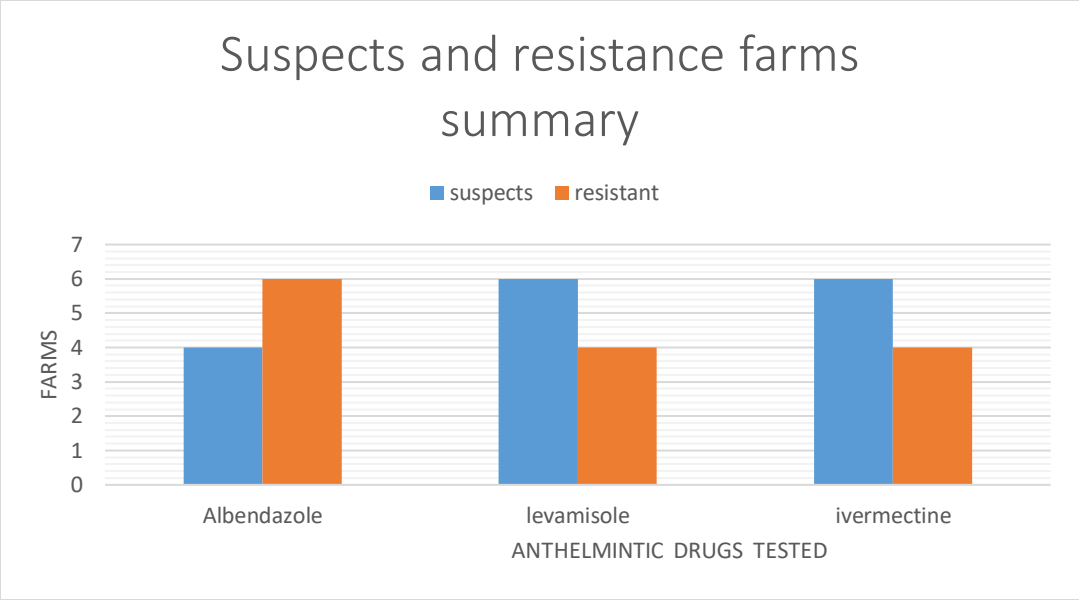



**Figure 4.6 : Summary of the number of farms where resistance was confirmed and suspected**

**Table 4.4: Percentage Faecal Egg Count Reduction (FECR%) and Lower 95% Confidence Level (CL) for the anthelmintics drugs tested for resistance in the selected 10 farms in Kasarani Sub-County.**

| | | Arithmetic mean egg per grams of faeces | | | | |
|---|---|---|---|---|---|---|
| Farms | Treatment | Pre-treatment (day 0) | Post-treatment (day 14) | FECR % | Lower 95% CL | Remarks |
| | Albendazole | 700 | 420 | 35.4 | 212 | Suspect |
| | Levamisole | 360 | 150 | 76.9 | 19.6 | Resistance |
| 1 | Ivermectin | 660 | 460 | 29.2 | 225 | Suspect |
| | Albendazole | 460 | 350 | 50 | 157 | Suspect |
| | Levamisole | 470 | 370 | 47.1 | 42.5 | Resistance |
| 2 | Ivermectin | 240 | 470 | 32.8 | 122 | Suspect |
| | Albendazole | 800 | 120 | 76 | 39.2 | Resistance |
| | Levamisole | 470 | 220 | 56 | 75.4 | Resistance |
| 3 | Ivermectin | 710 | 320 | 36 | 153 | Suspect |
| | Albendazole | 360 | 280 | 28.2 | 88.6 | Resistance |
| | Levamisole | 540 | 570 | -46.2 | 156.7 | Suspect |
| 4 | Ivermectin | 260 | 350 | 10.3 | 127.4 | Suspect |
| | Albendazole | 340 | 120 | 82.9 | -28.8 | Resistance |
| | Levamisole | 550 | 490 | 30 | 137 | Suspect |
| 5 | Ivermectin | 310 | 750 | -7.1 | 271.6 | Resistance |
| | Albendazole | 320 | 240 | 11.1 | 15.3 | Resistance |
| | Levamisole | 240 | 110 | 59.3 | 32 | Resistance |
| 6 | Ivermectin | 180 | 110 | 59.3 | -65 | Resistance |
| | Albendazole | 200 | 130 | 71.7 | 9.5 | Resistance |
| | Levamisole | 200 | 320 | 30.4 | 239.2 | Suspect |
| 7 | Ivermectin | 200 | 320 | 30.4 | 150.5 | Suspect |
| | Albendazole | 240 | 370 | 43.1 | 138 | Suspect |
| | Levamisole | 170 | 690 | -6.2 | 335 | Suspect |
| 8 | Ivermectin | 250 | 330 | 49.2 | 38.6 | Resistance |
| | Albendazole | 740 | 570 | 19.7 | -1.3 | Resistance |
| | Levamisole | 750 | 420 | 40.8 | 234 | Suspect |
| 9 | Ivermectin | 500 | 400 | 43.7 | -11.8 | Resistance |
| | Albendazole | 540 | 600 | 6.3 | 175 | Suspect |
| | Levamisole | 520 | 440 | 31.3 | 531 | Suspect |
| 10 | Ivermectin | 720 | 370 | 42.2 | 143 | Suspect |

**Faecal culture**

Pre- and post-treatment faecal cultures were done for each treatment group in each farm and three helminthes genera (*Haemonchus*, *Trichostrongylus* and *Oesophagostomum*) were identified as

described in Table 4.5. The most resistant nematode to the three anthelmintics tested in each farm was *Haemonchus contortus* with the highest percentage counts. The range of % $L_3$ counts at farm level were as follows; *H. contortus* (89%-100%), *Trichostrongylus sp*. (0%-12%), *Oesophagostomum* sp. (0% -5%).

**Table 4.5: The overall percentage differential counts of $L_3$ identified in the treated and control groups.**

| **Helminthes genera** | **Control** | **Albendazole treatment** | **Levamisole treatment** | **Ivermectin treatment** | **Remarks** |
|---|---|---|---|---|---|
| *Haemonchus* | 90% | 88% | 98% | 93% | Resistance |
| *Trichostrongylus* | 6% | 9% | 2% | 7% | Resistance |
| *Oesophagostomum* | 4% | 0% | 1% | 2% | Resistance to levamisole and ivermectin |

*Haemonchus contortus* exhibited resistance to all the anthelmintics tested in all the ten farms, while *Trichostrongylus* species exhibited resistance to all the anthelmintics tested in half of the farms. *Oesophagostomum* species were resistance to both levamisole and ivermectin in two different farms (Table 4.6).

**Table 4.6: Number of farms showing helminthes genera resistance to the tested anthelmintics**

| | **Number of farms showing helminthes genera resistance to the tested anthelmintics** | | |
|---|---|---|---|
| **Anthelmintic drugs** | ***Haemonchus contortus*** | ***Trichostrongylus* species** | ***Oesophagostomum* species** |
| Albendazole | 10 | 4 | 0 |
| Levamisole | 10 | 5 | 1 |
| Ivermectin | 10 | 4 | 1 |

## 5.0 DISCUSSION

High prevalence (99.6%) of gastrointestinal parasites reported in the present study corresponds to what was reported in Pakistan (94%) Ruhoollah *et al*., (2021) and in Ghana (98.2%) (Owusu *et al.,* 2016). However, a lower prevalence of 64% was reported in Nigeria (Maimadu *et al.,* 2020). The high prevalence of gastrointestinal parasites of sheep observed in the present study could be due to inadequate nutrition, poor management in terms of sanitation, overstocking and frequent exposure to contaminated communal grazing lands as 70% of the farmers practiced communal farming. This high prevalence limits sheep productivity and interferes with their well-being as farmers (70%) observed that the clinical signs of GI parasites in sheep were weight loss, diarrhoea, coughing and sneezing. Similar findings on prevalence rate were also reported in Nyandarua district (Maingi *et al.,* 2001), in Nigeria (Anene, 1994) and in Ethiopia (Yemisrach and Amenu 2017; Bikila *et al.,* 2013).

In this study, the prevalence of strongyle, tapeworm and coccidia was 72%, 11% and 49% respectively. However, there were no trematodes observed in the area. A slightly higher prevalence of strongyle (80%) was reported in Magadi division (Maichomo *et al.,* 2004). This contrasts with what was reported in Marsabit County where the prevalence of coccidia was 97% compared with that of strongyle at 6% Nakami *et al.,* (2015) and in Kisumu municipality where prevalence of strongyle and coccidia were 44% and 35% respectively (Kanyari *et al.,* 2009). The lower prevalence of tapeworm observed in the present study might be because of reduced egg dissemination in faeces from gravid proglottids as reported by Priyanka *et al.*, (2020). Absence of trematodes in the study area might be due to lack of intermediate host (snail) as well as natural water bodies.

The prevalence of gastro-intestinal parasites was similarly high in lambs (73%) and adults (72%) indicating that they are affected by same gastrointestinal parasites. The high prevalence in lambs and adults in the present study might be due to high susceptibility because of immunological

unresponsiveness in lambs and waned immunity in adults. This contrast with the findings in Rwanda (Mushonga *et al.,* 2018), Kenya (Ng'ang'a *et al.,* 2004) and Ethiopia (Getachew *et al.,* 2017) where prevalence was higher in lambs than adults. There were no differences in the prevalence of strongyle and coccidial infection between different sex of sheep, this agrees with the findings reported in Ethiopia (Getachew *et al.,* 2017), in Bangladesh (Podder, 2017) and in Rwanda (Mushonga *et al.,* 2018). Gastrointestinal parasites affected all sheep irrespective of age or sex especially in conditions where adults and lambs were mixed during grazing thus enhancing transmission of these parasites and established clinical infestation in the host as observed in the present study. In contrast other researchers have reported significant differences in gastrointestinal parasites infection between age groups and sex: Nyandarua District (Maingi *et al.,* 1997), Kajiado District (Ng'ang'a *et al.,* 2004), Kisumu municipality (Kanyari *et al.,* 2009) and Ethiopia (Abebe *et al.,* 2018).

Copro-culture results in the present study indicated that *Haemonchus contortus* was the most prevalent nematode (90%) agreeing with the findings reported in Naivasha (Gatongi *et al.,* 1998), Nyandarua Districts of Kenya (Maingi *et al.,* 1997) and India (Priyanka *et al.,* 2020). These findings differ with the findings in Kajiado District by Ng'ang'a *et al.,* (2004) where *Trichostrongylus* was more prevalent. In this study high prevalence of *Haemonchus contortus* could be attributed to its high fecundity and multiplies rapidly unlike other nematodes of sheep.

In the present study 70% of the sheep were kept under communal management systems, purely dependent on pasture (83%) with little nutritional supplementation, high stocking densities and limited veterinary consultation (13%). This agrees with the finding reported in Kenya (Kosgey *et al.,* 2008) and in Rwanda (Mushonga *et al.,* 2018). Majority (67%) of the farmers had very little knowledge on effects of helminthes on sheep production and to determine the level of infection they depended on clinical signs such as weight loss, diarrhoea, coughing and sneezing.

The present study showed that classes of anthelmintics available in the study area were benzimidazoles (albendazole), imidazothiazoles (levamisoles), and macrocyclic lactones (ivermectins) and the choice of anthelmintics to use was greatly depended on price (70%) and ease of administration (30%). This finding agrees with Nginyi, (2014) report that these three classes of broad-spectrum anthelmintics are found in most of the agricultural and veterinary drug stores in Kenya. These findings also agree with what was reported in low potential areas of Thika District (Githigia, 2000).

In the present study suppressive treatment was evident based on the frequency of treatment, farmers administer these anthelmintics whenever clinical signs appear (33%) and every 3 months (67%). However, administration of anthelmintics should not be a routine practice, but should be done strategically considering parasites epidemiology and confirmatory diagnosis (Ng'ang'a, 2002; Melaku *et al.*, 2013).

Farmers (67%) did not weigh their sheep, but rather depended on visual estimation of body weight and subsequent determination of volume of anthelmintics administered. Consequently, these might lead to under and over dosing which might enhance selection of worms resistant to these anthelmintics. Use of estimated body weight to determine dosage has been reported in Kenya (Maingi, 1996) and in Ethiopia (Melaku *et al*, 2013). Therefore, in the present study improper dosing (67%) and frequent alternation (73%) of these anthelmintics coupled with frequent deworming increased selection pressure for resistant nematodes and potentiated multiple anthelmintic resistance. Besides, majority of the farmers (83%) agreed that overuse of these anthelmintics leads to multiple anthelmintic resistance. These findings agree with previous studies which reported occurrence of multiple anthelmintic resistance (Maingi, 1996; Waruiru, 1997; Ng'ang'a *et al.*, 2010; Gakuya *et al.*, 2007; Melaku *et al.*, 2013; Swarnkar and Singh, 2010). In the present study, most farmers (87%) did

not seek for veterinary advice, but preferred to administer anthelmintics on their own. Working with sheep farmers in Ethiopia Seyoum *et al,* (2017) made a similar observation.

In the present study *Haemonchus contortus* was the most dominant nematode resistant to the anthelmintic drugs tested followed by *Trichostrongylus* species .The high resistance of *Haemonchus contortus* against the three classes of anthelmintics (benzimidazole, levamisole and macrocyclic lactones) has been reported in Kenya in previous studies (Mwamachi *et al,* 1995; Maingi, 1991; Waruiru *et al,* 1998; Gakuya *et al*, 2007).*Trichostrongylus* species were also resistance to albendazole, levamisole and ivermectin contrasting with what was reported by Gakuya *et al*, (2007), where *Trichostrongylus* species were resistance to levamisole and ivermectin but susceptible to albendazole. *Oesophagostomum* species were resistant to levamisole and ivermectin. The observed anthelmintics resistance is most likely due to; high treatment frequency, regular use of anthelmintics from different classes at the same time and inappropriate dosing as recorded in the present study.

## 6.0 CONCLUSION AND RECOMMENDATION

In conclusion, this study demonstrated high prevalence of gastrointestinal parasites infection in sheep despite the anthelmintics treatment. However, the intensity was low hence high prevalence doesn't necessitate treatment. Besides, sheep farmers lack adequate knowledge on anthelmintic usage, worm control strategies and challenge of anthelmintics resistance. Inappropriate dosing, frequent administration, frequent alternation and overuse of these anthelmintics by farmers in the present study could have potentiated multiple anthelmintic resistance. Resistance to albendazole was evident in more farms than to levamisole and ivermectin indicating reduced efficacy of albendazole due to overuse.

**Based on the findings the recommendations are as follows:**

1. **Farmers training**: The criteria used by farmers for dose determination was faulty hence there is need to educate the farmers to determine the dose of anthelmintics as per the heaviest animal, as the criteria used might results in under dosing which enhance development of anthelmintics resistance. Farmers should be encouraged to reduce treatment frequency and seek for proper planning by professionals.

   Awareness of worm management practices by farmers should be improved through integrated efforts of veterinary service providers, researchers, pharmaceutical companies and government involvement. The government should also put in place policies to control the distribution of anthelmintics and popularize alternative helminthes control programmes.

2. **Integrated control of helminthes:** farmers should not entirely depend on anthelmintics for treatment and control of helminthes rather should incorporate alternative approaches in order to maintain efficacy of the available anthelmintics. Strategic treatment based on professional advice upon screening of fecal egg output rather than at the end of prepatent period (whenever clinical signs

appear). Communal grazing enhances transmission of helminthes parasites, therefore, farmers should practice pasture management through rotational grazing, pasture resting and alternate grazing help minimize pasture contamination and limits continuous maintenance and progressive transmission of gastro-intestinal parasites to susceptible hosts. This also discourages over-reliance of the available classes of anthelmintics and delays development of multiple anthelmintic resistance.

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## APPENDICES

**Appendix 1: KPA survey to agricultural and veterinary drug stores sales persons in Kasarani.**

**A. Personal information (Biodata)**

Date----------Interviewers Name: Tangus Jesca

Name: ----------Mobile number and Email address---------- Gender: ----------

Practice/operation location GPS/GIS----------duration of service----------

**B. Knowledge on helminths and their control**

**1.** Do you sell anthelmintic in your agro-vet stores? 1. yes 2. no

. If yes which classes of anthelmintic drugs are available in your stores? 1.Albendazole 2. ivermectin 3.

levamisole.

3. Where do you purchase anthelmintics? 1. Pharmacy 2. clinic (veterinary) 2. Market 3. Drug suppliers

4. How do you keep anthelmintics? 1. Normal room temperature 2. Any temperature

5. which criteria do you use to determine the price of your anthelmintics? 1. Existing prices in the market 3. Number of sales made

6. Are you aware of anthelmintic resistance? 1.yes 2.no

**C. Attitude/perception about helminth control**

**7.** Helminthes are a major cause of poor performance in sheep in the area 1. Strongly disagree 2. Disagree 3. Agree 4. Strongly agree

**8.** Farmers in this area have adequate knowledge on effects of helminthes on sheep productivity 1. Strongly disagree 2. Disagree 3. Agree 4. Strongly agree

9. Farmers in this area have adequate knowledge on treatment of helminthes in sheep 1. Strongly disagree 2. Disagree 3. Agree 4. Strongly agree

10. Farmers in this area consult vets when confronted with suspected helminth problems 1. Not at all 2. sometimes 3. most of the times 4. all the time

11. Do you receive complaints from farmers on ineffectiveness of anthelmintics? 1. yes 2. no

12. If yes, which anthelmintics? 1. Benzimidazoles (BZ)- Albendazole 2. Macrocyclic lactones (ML)- ivermectin 3. Imidazothiazoles/Tetrahydropyrimidines (LV)- levamisole.

13. Why do you think anthelmintics are not effective? 1. dosing inappropriately 2. class of anthelmintics 3. Usage rate

14. What advice do you give to farmers concerning anthelmintic resistance?

**D. Practices on helminth control**

15. Which class of anthelmintics is frequently bought by farmers? 1. Albendazole 2. Ivermectin 3. Levamisole

16. How do farmers select dewormers? 1. Cost (cheap) 2. Appearance (color) 3. Known effectiveness 4. Advice from Veterinarians

17. What's the rate at which farmers/veterinarians buy the anthelmintics in your agro-vet? 1. Bulk 2. Small Quantities?

18. How often do farmers visit your agro-vet store for anthelmintics? 1. Monthly 2. Every three months 3. Towards the onset of rainy or dry season

19. Do you advise the farmer on drug administration and dosage determination? 1. yes/ 2. no

20. If yes, how? Weight of each Sheep 2. Use the weight of the heaviest sheep 3. Visual Estimation

21. Do farmers rotate anthelmintics? 2. yes 2. no

22. If yes, why? 1. Ineffectiveness of previous anthelmintics? 2. it's a routine

**Appendix 2: a survey questionnaire on knowledge, practice and attitude towards treatment and control of gastrointestinal parasites in sheep, to farmers**

**A. Personal information (biodata):**

Name and contact: ----------Age: ----------Gender: ----------

Farm location---------- Flock size----------sheep breed----------

**B. Knowledge on helminths and their control**

**1.**Which sheep production system do you practice? 1. Open 2. Mixed 3. Communal

2. Is your feeding regime purely on pasture or you supplement 1. yes 2. no

3. How do you Handle new sheep/flock? 1. Mix with Other Flock 2. Isolate from Rest of the Flock

4. Are you aware about sheep worms? 1. yes 2.no

5. Which condition indicate that sheep have worms? 1. Loss of body condition 2. Diarrhea 3. Coughing 4. Inappetance

6. Which dewormers do you know 1. Albendazole alone, 2. Levamisole alone 3. Ivermectin alone

7. How have sheep worms affected the production of your animals? 1. No effects 2. Loss of weight 3. Increase cost of treatment

8. What are the changes after deworming? 1. Improvement 2. No changes observed 3. No follow up made.

9. Are the dewormers effective? 1. yes 2. no

**C. Attitude/perception about helminths**

**10.**Helminths are a major cause of poor performance in sheep in the farm 1. strongly disagree 2. disagree 3. agree 4. strongly agree

11.when confronted with suspected helminth problems do you consult experts? 1. Not at all 2. sometimes 3. most of the times 4. all the time

12.Nilzan is the best dewormer, others are not effective 1. Strongly disagree 2. Disagree 3. Agree 4. Strongly agree

13. The more times I deworm in a year, the better the sheep perform 1. Strongly disagree 2. Disagree 3. Agree 4. Strongly agree

14.The larger the volume of dewormer given, the more worms it clears 1. Strongly disagree 2. Disagree 3. Agree 4. Strongly agree

15.Injectable dewormers are better than drenches 1. Strongly disagree 2. Disagree 3. Agree 4. Strongly agree

16. If a dewormer is used for a long time or lower volume, worms get used to it 1. Strongly disagree 2. Disagree 3. Agree 4. Strongly agree

17. Dewormer I buy myself is of better quality than administered by local vet 1. Strongly disagree 2. Disagree 3. Agree 4. strongly agree

**D. Treatment and control of helminths**

**18.** Do you deworm your sheep? 1.yes 2. no

19. Which criteria do you use to select a dewormer? 1. Cost (cheap) 2. Appearance (colour) 3. Familiarity/ease of application 4. Veterinarian's advice 5. Known effectiveness

20. Where do you buy dewormers? 1. Nearby Agro-Vets 2. Market 3. Veterinary Clinic/Veterinarians

21. How do you determine the amount of dewormer to administer? 1. Weight of the animal 2. Weight of the heaviest sheep 3. Prescription 4. Visual estimation

22. How often do you deworm? 1. Every 3 Months 2. Twice a Year 3. Once a Year 4. When animal is sick.

23.Is your deworming regime affected by season? 1. Deworm at the onset of rain 2. Dry Season 3. throughout the Year

24.How can you tell you have administered the right dose? 1. Problem of overdose (Diarrhea, lack of appetite, Death, No Problem) 2. Problem of under dose (No problem, not sure, failure of the animal to respond appropriately) 3. Can't tell

25.Do you rotate dewormers? 1.yes 2.no

26.After how long do you rotate? 1. Annually 2. Twice Every Year 3. Whenever I feel it's necessary

27.Any other control strategy you use?

**Appendix 3: A survey questionnaire on knowledge, practice and attitude towards treatment and control of gastrointestinal parasites of sheep, for veterinary service providers in Kasarani**

**A. Personal information (biodata):**

Date: ---------- Interviewers Name: Tangus Jesca

Name: ----------Age: ----------Gender: ----------level of education: ----------

Practice/operation location GPS/GIS----------duration of service----------

**B. Knowledge on helminths and their control**

**1.** Which are the common Sheep farming systems practiced this area?1. Open 2. Mixed 3. Communal

2. Is helminths infestation in a major challenge in this area? 1. yes 2. no

3. If yes, what are the common clinical signs raised by the farmers in regard to Helminths infestation? 1. Loss of Body condition 2. Diarrhea 3. Coughing and sneezing 4. Anorexia 5. Stunted growth

4. Which classes of anthelmintics are available in your area of jurisdiction? 1.Benzimidazoles 2. Macrocyclic Lactones

3. Imidazothiazoles/Tetrahydro pyrimidines.

5. Is there a need/regulation to change a dewormer after a period of time? 1. yes 2. no?

6. If yes, how often? 1. Every year 2. every 2-3 years 3. Twice every year

7. Who is to source for anthelmintic drugs for sheep? 1. Farmer, 2. Veterinarians 3. County veterinary department

8. Recommend a source for anthelmintic drugs**?**1. Veterinary Clinic 2. Market 3. Drug suppliers 4. Pharmacy stores

**C. Attitude/perception about helminthes control and treatment**

**9.** farmers in this area consult experts when confronted with helminths problem 1. Not at all 2. Sometimes 3. Most of the

time 4. All the time

10. Have you faced a challenge of Anthelmintic Resistance? 1. yes 2. no

11. If yes, what do you recommend as the way forward to solve the challenge of anthelmintic resistance (AR)?

**D. Treatment and control of helminthes parasites**

12. How do you administer the anthelmintics? 1. Personally 2. Prescribe for The Farmer to Administer?

13. How do you determine the dose to administer? 1. Weight of individual animal 2. Weight of the heaviest animal in a group 3. Visual judgement/estimation,

14. How often do farmers deworm? 1.Every 3 Months 2. Twice A Year 3. Once A Year 4. When animal is tick.

15. Do you advise farmers to rotate anthelmintics drugs? 1. yes 2. no

16. If So, after how long do you recommend to alternate? 1. Annually, 2. Twice every Year, 3. When the previous anthelmintic is no longer effective

17. is there other helminth control strategy practice in this area? 1. yes 2. no

18. if yes which strategy (s)?

**Appendix 4: List of farmers interviewed in KPA survey, their gender, farm location and flock size** in Kasarani**.**

| Name | Gender | Location | Flock size |
|---|---|---|---|
| Miriam Nyawera | Female | Ruai | 13 |
| Mary Wangoi | Female | Shujaa | 28 |
| Mrs. Maina | Female | Kamulu | 70 |
| Raphael Muturi | Male | Kipawa | 45 |
| John Pislei | Male | Ruai | 60 |
| Pastor James | Male | Ruai | 65 |
| Paul Ndirango | Male | Ruai | 68 |
| John Matenge | Male | Kamulu | 30 |
| William Sinigi | Male | Ruai | 80 |
| Elias Sinigi | Male | Ruai | 108 |
| Reuben Maa | Male | Ruai | 70 |
| Stanley Taiko | Male | Ruai | 100 |
| Timo Maya | Male | Ruai | 100 |
| Abraham Oleteiya | Male | Utawala | 64 |
| John Kimer | Male | Utawala | 92 |
| Gabriel Parken | Male | Utawala | 70 |
| Jeremiah Olemutua | Male | Utawala | 85 |
| Emanuel Koilel | Male | Utawala | 75 |
| Jeremiah Mpooke | Male | Ruai | 150 |
| David Pulei | Male | Ruai | 160 |
| Josphat Maya | Male | Ruai | 130 |
| Tomie Koshooi | Male | Ruai | 145 |
| Nelson Tekei | Male | Ruai | 89 |
| Leonard Maya | Male | Ruai | 160 |
| Moses Pulei | Male | Utawala | 86 |
| Joseph Tumanka | Male | Utawala | 90 |
| William Maya | Male | Ruai | 170 |
| Ndabis | Male | Ruai | 150 |
| Moses Mpooke | Male | Ruai | 113 |
| Gideon Kooshoi | Male | Ruai | 170 |

**Appendix 5: List of Agricultural and veterinary drug stores sampled in KPA, their location and duration of service** in Kasarani**.**

| **Name** | **Location** | **Duration of practice** |
|---|---|---|
| Bypass Vet-Agro | Ruai | 3 years |
| Oryx | Ruai | More than 20 years |
| Farm Pride | Ruai | 2 years |
| Morden | Ruai | 5 years |
| Morden Agro Care | Ruai | 6 years |
| Lishe Bora | Ruai | 5 years |
| Feeds Vet/ Supplies | Ruai | 2 years |
| Fauna Agrovet | Kamulu | 1 year |
| Jumbo Agro Dealers | Ruai | 10 years |
| Oasis | Ruai | 7 years |
| Ruai Agrovet | Ruai | 3 years |
| Mwiki Agrovet | Mwiki | 2 years |
| Maziwa Vet Agency | Ruai | 4 years |
| Kasarani Agro Chem | Kasarani | 2 years |
| Pembe Agrovet | Ruai | 5 years |
| Njawa | Kamulu | 3 years |
| Acacia | Kamulu | 6 years |
| Kamulus | Kamulu | 5 years |
| Lishe Bora | Kamulu | 2 years |
| Farm Pride Agrovet | Makongeni | 3 years |
| Nafuu Agrovet | Ruai | 4 years |
| Ngombe Agrovert | Kamulu | 1 year |
| Kilimo Agrovet | Ruai | 6 years |

**Appendix 6: List of Veterinary service providers interviewed in KPA survey, their biodata and location of practice in Kasarani.**

| Name | Location | Level of education | Age | Gender | Title |
|---|---|---|---|---|---|
| Margret Wanjiku | Ruai | Degree | 36 | Female | Veterinary surgeon |
| Nancy Mulwa | Nairobi | Degree | 28 | Female | Veterinary surgeon |
| Ceciline Wanjiku | Ruai | Degree | 28 | Female | Animal health assistant |
| Moses Kiambuthi | Ruai | Degree | 35 | Male | Veterinary surgeon |
| Wilson Matemo | Ruai | Degree | 56 | Male | Veterinary surgeon |
| Samuel Njogu | Ruai | Degree | 34 | Male | Veterinary surgeon |
| Veronicah Nduda | Ruai | Certificate | 34 | Female | Animal health assistant |
| Grace Waweru | Ruai | Certificate | 30 | Female | Animal health assistant |
| Mercy Nyawa | Ruai | Certificate | 29 | Female | Animal health assistant |
| Maina peter | Kamulu | Certificate | 25 | Male | Animal health assistant |
| Evans Mwangi | Ruai | Certificate | 32 | Male | Animal health assistant |
| Yusuf Odhiambo | Nairobi | Certificate | 34 | Male | Animal health assistant |
| Cyrus Mwangi | Nairobi | Certificate | 32 | Male | Animal health assistant |
| Janet Cheptoo | Ruai | Diploma | 30 | Female | Animal health assistant |
| Judith Barasa | Ruai | Diploma | 54 | Female | Animal health assistant |
| Roselyne Maina | Ruai | Diploma | 29 | Female | Animal health assistant |
| Martin Bedan | Kasarani | Diploma | 32 | Male | Animal health assistant |
| Joseph Malasi | Utawala | Diploma | 34 | Male | Animal health assistant |
| Benson Munyoki | Acacia | Diploma | 27 | Male | Animal health assistant |
| Japhet Mbogera | Nairobi | Diploma | 37 | Male | Animal health assistant |
| Joyce Mwikali | Ruai | Diploma | 34 | Female | Animal health assistant |
| Moses Mwangi | Ruai | Diploma | 40 | Male | Animal health assistant |
| Esther Nyaboke | Kasarani | Degree | 32 | Female | Veterinary surgeon |
| Christian Okech | Nairobi | Degree | 32 | Male | Veterinary surgeon |

**Appendix 7: Responses on Knowledge, Practice and Attitude towards treatment and control of helminthes in sheep by farmers in Kasarani.**

| Attributes | Responses | Frequency | Percentage |
|---|---|---|---|
| Farming system | Open | 1 | 3.33% |
| | Mixed | 8 | 26.67% |
| | Communal | 21 | 70.00% |
| Feeding regime | Supplement | 5 | 16.67% |
| | No supplement | 25 | 83.33% |
| Handling of new flock | Mix new sheep | 28 | 93.33% |
| | Don't mix new sheep | 2 | 6.67% |
| Knowledge about worms | Helminthes awareness | 30 | 100.00% |
| Signs of helminthes infestation | Loss of body condition | 4 | 13.33% |
| | Diarrhoea | 3 | 10.00% |
| | Coughing and sneezing | 2 | 6.67% |
| | All signs | 21 | 70.00% |
| Known dewormer | Albendazole alone | 13 | 43% |
| | Ivermectin alone | 8 | 27% |
| | Levamisole alone | 9 | 30% |
| Effects of worms on sheep production | Weight loss | 5 | 17% |
| | High cost of treatment | 5 | 17% |
| | Both | 20 | 66% |
| Effectiveness of anthelmintics | Improvement | 29 | 96.67% |
| | No changes | 1 | 3.33% |
| Helminthes major cause of poor performance in sheep | Strongly disagree | 0 | 0.00% |
| | Disagree | 2 | 6.67% |
| | Agree | 1 | 3.33% |
| | Strongly agree | 27 | 90.00% |
| Consult experts on suspected helminthosis | Not at all | 26 | 86.67% |
| | Sometimes | 1 | 3.33% |
| | Most of the time | 0 | 0.00% |
| | All the time | 3 | 10.00% |
| Nilzan is the best dewormer, others not effective | Strongly disagree | 1 | 3.33% |
| | Disagree | 2 | 6.67% |
| | Agree | 12 | 40.00% |
| | Strongly agree | 15 | 50.00% |
| | Strongly disagree | 2 | 6.67% |

| | | | |
|---|---|---|---|
| Deworming more times, better sheep performance | Disagree | 2 | 6.67% |
| | Agree | 13 | 43.33% |
| | Strongly agree | 13 | 43.33% |
| Larger volume of dewormers clears more worms | Strongly disagree | 13 | 43.33% |
| | Disagree | 7 | 23.33% |
| | Agree | 9 | 30.00% |
| | Disagree | 1 | 3.33% |
| Injectables better than drenches | Strongly disagree | 9 | 30.00% |
| | Disagree | 3 | 10.00% |
| | Agree | 13 | 43.33% |
| | Strongly agree | 5 | 16.67% |
| Anthelmintic overuse cause resistance | Strongly disagree | 2 | 6.67% |
| | Disagree | 1 | 3.33% |
| | Agree | 2 | 6.67% |
| | Strongly agree | 25 | 83.33% |
| Better dewormer bought by farmer than administered by vet | Strongly disagree | 5 | 16.67% |
| | Disagree | 2 | 6.67% |
| | Agree | 6 | 20.00% |
| | Strongly agree | 17 | 56.67% |
| Deworm sheep | Yes | 30 | 100.00% |
| Anthelmintic selection criteria | Low price | 9 | 30% |
| | Ease of application | 21 | 70.00% |
| Source of dewormers | Veterinary suppliers | 3 | 10.00% |
| | Nearby agro-vet | 27 | 90.00% |
| Dosage determination | Weight of the animal | 1 | 3.33% |
| | Prescription | 9 | 30.00% |
| | Visual estimation | 20 | 66.67% |
| Frequency of deworming | Every 3 months | 20 | 66.67% |
| | When the animal i sick | 9 | 30.00% |
| Deworming season | Onset of rain | 7 | 23.33% |
| | Throughout the year | 23 | 76.67% |
| Over dose problem | Severe diarrhoea | 2 | 6.67% |
| | No problem | 20 | 66.67% |
| | Can't tell | 8 | 26.67% |
| Under dose problem | No problem | 6 | 20.00% |
| | Doubtful | 10 | 33.33% |
| | Fail to cure | 14 | 46.67% |
| Anthelmintic rotation | Twice every year | 6 | 20.00% |
| | Whenever need be | 22 | 73.33% |
| | Annually | 2 | 6.67% |

| | | | |
|---|---|---|---|
| Other helminthes control strategies | Yes | 0 | 0.00% |
| | No | 30 | 100.00% |

**Appendix 8: Responses on Knowledge, Practice and Attitude towards treatment and control of helminthes in sheep by agricultural and veterinary drug stores sales persons in Kasarani**

| Attributes | Responses | Frequency | Percentage |
|---|---|---|---|
| Available anthelmintics | Albendazole, ivermectin and levamisole | 22 | 95.00% |
| | Albendazole alone | 1 | 5.00% |
| Anthelmintics source | Veterinary suppliers | 23 | 100.00% |
| Anthelmintic storage | Room temperature | 21 | 91.00% |
| | Anywhere | 2 | 9.00% |
| Price determination | Market price | 21 | 91.30% |
| | Frequency of sale | 2 | 8.70% |
| AR awareness | Yes | 19 | 82.61% |
| | No | 4 | 17.39% |
| Helminths major cause of poor performance in sheep | Strongly disagree | 0 | 0.00% |
| | Disagree | 2 | 8.70% |
| | Agree | 11 | 47.83% |
| | Strongly agree | 10 | 43.48% |
| Farmers know effects of helminthes | Strongly disagree | 1 | 4.35% |
| | Disagree | 3 | 13.04% |
| | Agree | 12 | 52.17% |
| | Strongly agree | 7 | 30.44% |
| Farmers know helminthes treatment | Strongly disagree | 4 | 17.39% |
| | Disagree | 6 | 26.09% |
| | Agree | 10 | 43.48% |
| | Strongly agree | 3 | 13.04% |
| Farmers consult experts | Not at all | 3 | 13.04% |
| | Sometimes | 9 | 39.13% |
| | Most of the time | 4 | 17.39% |
| | All the time | 7 | 30.43% |

| | | | |
|---|---|---|---|
| Complains on dewormers ineffectiveness | Yes | 16 | 69.57% |
| | No | 7 | 30.43% |
| Ineffective dewormer | Albendazole | 14 | 60.87% |
| | Ivermectin | 0 | 0.00% |
| | Levamisole | 2 | 4.35% |
| | All of the above | 7 | 30.43% |
| Reason for ineffectiveness | Improper dosing | 3 | 13.04% |
| | Dewormer type | 2 | 8.70% |
| | Overuse | 18 | 78.26% |
| Advice on AR | Alternate dewormers | | |
| | Regulate treatment | | |
| | Consult expert | | |
| Dewormers frequently bought | Albendazole | 13 | 56.52% |
| | Levamisole | 6 | 26.09% |
| | Albendazole, ivermectin, levamisole | 4 | 17.39% |
| Selection criteria | Price | 9 | 34.78% |
| | Evidence of efficacy | 7 | 34.78% |
| | Vets advice | 7 | 30.43% |
| Rate of buying dewormers by farmers | Bulk | 2 | 8.70% |
| | Small quantities | 21 | 91.30% |
| Rate of visit by farmers | Monthly | 4 | 21.74% |
| | Every three months | 4 | 21.74% |
| | Onset of rain | 2 | 8.70% |
| | Throughout | 10 | 47.83% |
| Advice on administration | Yes | 23 | 100.00% |
| Dose determination | Weight of individual sheep | 9 | 39.13% |
| | Weight of heaviest the sheep | 0 | 0.00% |
| | Visual judgement | 14 | 60.87% |
| Farmers rotate dewormers | Yes | 14 | 60.87% |

| | | | |
|---|---|---|---|
| | No | 9 | 39.13% |
| Reason for rotation | Ineffectiveness of previous dewormer | 16 | 69.57% |
| | Routine | 7 | 30.43% |

**Appendix 9: Responses on Knowledge, Practice and Attitude towards sheep's helminthes, treatment and control by veterinary service providers in Kasarani**

| Attributes | Responses | Frequency | Percentage |
|---|---|---|---|
| Farming system | Open | 2 | 8.33% |
| | Mixed | 12 | 50.00% |
| | Communal | 10 | 41.67% |
| Helminthosis is a big problem | True | 18 | 75.00% |
| | False | 6 | 25.00% |
| Clinical signs of helminthosis | Loss of body condition | 4 | 16.67% |
| | Diarrhoea | 2 | 8.33% |
| | Both | 18 | 75.00% |
| Classes of anthelmintics available | Benzimidazoles | 6 | 25.00% |
| | Macrocyclic lactones | 2 | 8.33% |
| | Imidazothiazole | 4 | 16.67% |
| | All | 12 | 50.00% |
| Regulation to alternate dewormers | Yes | 18 | 75.00% |
| | No | 6 | 25.00% |
| Need to alternate dewormers | Yes | 24 | 100.00% |
| | No | 0 | 0.00% |
| Best anthelmintic rotation period | 2-3 years | 4 | 16.67% |
| | Twice a year | 12 | 50.00% |
| | Once a year | 8 | 33.33% |
| Person to source dewormers | No idea | 3 | 12.50% |
| | Farmer | 12 | 50.0% |
| | Veterinarian | 9 | 37.50% |
| Recommended source of dewormers | Veterinary suppliers | 9 | 37.50% |
| | Open air market | 3 | 12.50% |
| | Agro-vets | 8 | 33.33% |

| | | | |
|---|---|---|---|
| | Drug pharmacy | 4 | 16.67% |
| Farmer consults | Not at all | 0 | 0.00% |
| | Sometimes | 15 | 62.50% |
| | Most of the time | 6 | 25.00% |
| | All the time | 3 | 12.50% |
| Challenge of AR | Yes | 16 | 66.67% |
| | No | 8 | 33.33% |
| AR solution | Rotate dewormer | | |
| | Strategic deworming | | |
| | Farmers education | | |
| | Vets to administer | | |
| Anthelmintic administration | Personally | 9 | 37.50% |
| | Prescribe to farmers | 15 | 62.50% |
| Dose determination | Weight of the animal | 14 | 58.33% |
| | Weight of the heaviest animal | 3 | 12.50% |
| | Visual estimation | 7 | 29.17% |
| Deworming frequency | Every three months | 18 | 75.00% |
| | Twice a year | 3 | 12.50% |
| | When animal is sick | 3 | 12.50% |
| Advice on anthelmintic rotation | Yes | 24 | 100.00% |
| Other helminthes control strategies | Yes | 0 | 0.00% |
| | No | 24 | 100.00% |

**Appendix 10: Prevalence of gastro-intestinal parasites infection in sheep in the selected 30 farms in Kasarani**

| Farms | Sheep sampled | Strongyle | Tapeworms | Coccidia |
|---|---|---|---|---|
| Farm1 | 100 | (78)78% | (7)7% | (62)62% |
| Farm2 | 13 | (4)31% | (5)38% | (0)0% |
| Farm3 | 59 | (30)51% | (5)8% | (29)49% |
| Farm4 | 63 | (46)72% | (4)6% | (55)86% |
| Farm5 | 12 | (3)25% | (0)0% | (7)58% |
| Farm6 | 25 | (7)28% | (0)0% | (14)56% |
| Farm7 | 121 | (100)83% | (2)2% | (47)39% |
| Farm8 | 80 | (53)66% | (10)13% | (52)65% |
| Farm9 | 21 | (12)57% | (3)14% | (11)52% |
| Farm10 | 88 | (72)82% | (3)3% | (13)15% |
| Farm11 | 100 | 77)77% | (8)8% | (12)12% |
| Farm12 | 40 | (39)98% | (9)8% | (19)48% |
| Farm13 | 95 | (85)89% | (5)5% | (37)39% |
| Farm14 | 100 | (87)87% | (3)3% | (49)49% |
| Farm15 | 86 | (68)79% | (7)8% | (45)52% |
| Farm16 | 50 | (39)78% | (7)14% | (22)44% |
| Farm17 | 100 | (58)58% | (11)11% | (30)30% |
| Farm18 | 100 | (83)83% | (10)10% | (35)35% |
| Farm19 | 32 | (17)53% | (5)16% | (16)50% |

| | | | | |
|---|---|---|---|---|
| Farm20 | 65 | (53)82% | (1)2% | (37)57% |
| Farm21 | 54 | (42)78% | (1)2% | (35)65% |
| Farm22 | 32 | (14)44% | (1)3% | (14)44% |
| Farm23 | 29 | (14)48% | (1)3% | (13)45% |
| Farm24 | 31 | (10)32% | (0)0% | (16)52% |
| Farm25 | 25 | (10)40% | (0)0% | (14)56% |
| Farm26 | 42 | (14)33% | (3)7% | (5)12% |
| Farm27 | 19 | (18)95% | (0)0% | (13)68% |
| Farm28 | 19 | (16)84% | (0)0% | (7)37% |
| Farm29 | 18 | (17)94% | (0)0% | (9)50% |
| Farm30 | 23 | (22)96% | (0)0% | (11)48% |
| **Overall prevalence** | **1642** | **(1188)72%** | **(115)7%** | **(805)49%** |

**Appendix 11: Intensity of gastrointestinal parasites infection in sheep in the selected 30 farms**

| **Farms** | **Number sampled** | **Strongyle eggs** | **Tapeworm eggs** | **Coccidial oocyst** |
|---|---|---|---|---|
| Farm1 | 100 | 530 | 24 | 477 |
| Farm2 | 13 | 46 | 100 | 0 |
| Farm3 | 59 | 314 | 207 | 531 |
| Farm4 | 63 | 470 | 41 | 2034 |
| Farm 5 | 12 | 125 | 0 | 325 |
| Farm 6 | 25 | 284 | 0 | 252 |
| Farm 7 | 121 | 468 | 5 | 94 |
| Farm 8 | 80 | 558 | 81 | 343 |
| Farm 9 | 21 | 176 | 71 | 557 |
| Farm 10 | 88 | 538 | 160 | 267 |
| Farm 11 | 100 | 480 | 95 | 43 |
| Farm 12 | 40 | 1495 | 128 | 330 |
| Farm 13 | 95 | 819 | 31 | 122 |
| Farm14 | 100 | 497 | 14 | 166 |
| Farm15 | 86 | 441 | 49 | 278 |
| Farm16 | 50 | 164 | 22 | 120 |
| Farm17 | 100 | 145 | 0 | 112 |
| Farm18 | 100 | 479 | 173 | 137 |
| Farm19 | 32 | 116 | 106 | 228 |
| Farm20 | 65 | 537 | 26 | 308 |
| Farm21 | 54 | 704 | 2 | 669 |
| Farm22 | 32 | 66 | 13 | 300 |
| Farm23 | 29 | 76 | 14 | 255 |
| Farm24 | 31 | 45 | 0 | 116 |
| Farm25 | 25 | 68 | 0 | 176 |
| Farm26 | 42 | 32 | 0 | 7 |
| Farm27 | 19 | 1395 | 0 | 611 |
| Farm28 | 19 | 947 | 0 | 426 |
| Farm29 | 18 | 1561 | 0 | 267 |
| Farm30 | 23 | 1296 | 0 | 143 |
| Overall intensity | **1642** | 486 | 60 | 340 |

**Appendix12: Sheep's helminthes genera identified in the selected 30 farms in Kasarani**

| **Farms** | ***Haemonchus*** | ***Trichostrongylus*** | ***Oesophagostomum*** |
|---|---|---|---|
| Farm1 | 100% | 0% | 0% |
| Farm2 | 98% | 1% | 1% |
| Farm3 | 57% | 0% | 43% |
| Farm4 | 79% | 21% | 0% |
| Farm5 | 89% | 11% | 0% |
| Farm6 | 77% | 0% | 23% |
| Farm7 | 88% | 9% | 3% |
| Farm8 | 82% | 8% | 10% |
| Farm9 | 89% | 11% | 0% |
| Farm10 | 95% | 5% | 0% |
| Farm11 | 91% | 9% | 0% |
| Farm12 | 98% | 0% | 2% |
| Farm13 | 84% | 12% | 4% |
| Farm14 | 89% | 11% | 0% |
| Farm15 | 96% | 0% | 4% |
| Farm16 | 98% | 2% | 0% |
| Farm17 | 88% | 12% | 0% |
| Farm18 | 91% | 4% | 5% |
| Farm 19 | 92% | 3% | 5% |
| Farm20 | 100% | 0% | 0% |
| Farm21 | 93% | 2% | 5% |
| Farm22 | 88% | 0% | 12% |
| Farm23 | 80% | 6% | 14% |
| Farm24 | 87% | 13% | 0% |
| Farm25 | 97% | 0% | 3% |
| Farm26 | 94% | 6% | 0% |
| Farm27 | 98% | 0% | 2% |
| Farm28 | 89% | 1% | 10% |
| Farm29 | 99% | 1% | 0% |
| Farm30 | 87% | 3% | 10% |
| Overall spectrum | 90% | 5% | 5% |

**Appendix 13: Percentage differential counts of $L_3$ identified in the treated and control groups per farm in Kasarani.**

| Farm | Treatment | Helminthes genera | Pre-treatment | Post-treatment |
|---|---|---|---|---|
| | | *Haemonchus* | 88% | 96% |
| | | *Trichostrongylus* | 9% | 4% |
| | Albendazole | *Oesophagostomum* | 3% | 0% |
| | | *Haemonchus* | 88% | 96% |
| | | *Trichostrongylus* | 9% | 3% |
| | Levamisole | *Oesophagostomum* | 3% | 1% |
| | | *Haemonchus* | 88% | 100% |
| | | *Trichostrongylus* | 9% | 0% |
| | Ivermectin | *Oesophagostomum* | 3% | 0% |
| | | *Haemonchus* | 88% | 88% |
| | | *Trichostrongylus* | 9% | 9% |
| 1 | Control | *Oesophagostomum* | 3% | 3% |
| | | *Haemonchus* | 89% | 92% |
| | | *Trichostrongylus* | 11% | 8% |
| | Albendazole | *Oesophagostomum* | 0% | 0% |
| | | *Haemonchus* | 89% | 96% |
| | | *Trichostrongylus* | 11% | 4% |
| | Levamisole | *Oesophagostomum* | 0% | 0% |
| | | *Haemonchus* | 89% | 90% |
| | | *Trichostrongylus* | 11% | 10% |
| | Ivermectin | *Oesophagostomum* | 0% | 0% |
| | | *Haemonchus* | 89% | 89% |
| | | *Trichostrongylus* | 11% | 11% |
| 2 | Control | *Oesophagostomum* | 0% | 0% |
| | | *Haemonchus* | 95% | 99% |
| | | *Trichostrongylus* | 5% | 1% |
| | Albendazole | *Oesophagostomum* | 0% | 0% |
| | | *Haemonchus* | 95% | 98% |
| | | *Trichostrongylus* | 5% | 2% |
| | Levamisole | *Oesophagostomum* | 0% | 0% |
| | | *Haemonchus* | 95% | 96% |
| | | *Trichostrongylus* | 5% | 4% |
| | Ivermectin | *Oesophagostomum* | 0% | 0% |
| | | *Haemonchus* | 95% | 95% |
| | | *Trichostrongylus* | 5% | 5% |
| 3 | Control | *Oesophagostomum* | 0% | 0% |
| | | *Haemonchus* | 91% | 93% |
| | | *Trichostrongylus* | 9% | 7% |
| 4 | Albendazole | *Oesophagostomum* | 0% | 0% |

| | | | | |
|---|---|---|---|---|
| | | *Haemonchus* | 91% | 95% |
| | | *Trichostrongylus* | 9% | 5% |
| | Levamisole | *Oesophagostomum* | 0% | 0% |
| | | *Haemonchus* | 91% | 100% |
| | | *Trichostrongylus* | 9% | 0% |
| | Ivermectin | *Oesophagostomum* | 0% | 0% |
| | | *Haemonchus* | 91% | 91% |
| | | *Trichostrongylus* | 9% | 9% |
| | Control | *Oesophagostomum* | 0% | 0% |
| | | *Haemonchus* | 89% | 100% |
| | | *Trichostrongylus* | 11% | 0% |
| | Albendazole | *Oesophagostomum* | 0% | 0% |
| | | *Haemonchus* | 98% | 97% |
| | | *Trichostrongylus* | 11% | 3% |
| | Levamisole | *Oesophagostomum* | 0% | 0% |
| | | *Haemonchus* | 89% | 94% |
| | | *Trichostrongylus* | 11% | 6% |
| | Ivermectin | *Oesophagostomum* | 0% | 0% |
| | | *Haemonchus* | 89% | 89% |
| | | *Trichostrongylus* | 11% | 11% |
| 5 | Control | *Oesophagostomum* | 0% | 0% |
| | | *Haemonchus* | 96% | 100% |
| | | *Trichostrongylus* | 0% | 0% |
| | Albendazole | *Oesophagostomum* | 4% | 0% |
| | | *Haemonchus* | 96% | 100% |
| | | *Trichostrongylus* | 0% | 0% |
| | Levamisole | *Oesophagostomum* | 4% | 0% |
| | | *Haemonchus* | 96% | 98% |
| | | *Trichostrongylus* | 0% | 0% |
| | Ivermectin | *Oesophagostomum* | 4% | 2% |
| | | *Haemonchus* | 96% | 96% |
| | | *Trichostrongylus* | 0% | 0% |
| 6 | Control | *Oesophagostomum* | 4% | 4% |
| | | *Haemonchus* | 98% | 100% |
| | | *Trichostrongylus* | 2% | 0% |
| | Albendazole | *Oesophagostomum* | 0% | 0% |
| | | *Haemonchus* | 98% | 4% |
| | | *Trichostrongylus* | 2% | 0% |
| | Levamisole | *Oesophagostomum* | 0% | 0% |
| | | *Haemonchus* | 98% | 100% |
| | | *Trichostrongylus* | 2% | 0% |
| | Ivermectin | *Oesophagostomum* | 0% | 0% |
| | | *Haemonchus* | 98% | 98% |
| | | *Trichostrongylus* | 2% | 2% |
| 7 | Control | *Oesophagostomum* | 0% | 0% |

| | | | | |
|---|---|---|---|---|
| | | *Haemonchus* | 88% | 98% |
| | | *Trichostrongylus* | 12% | 2% |
| | Albendazole | *Oesophagostomum* | 0% | 0% |
| | | *Haemonchus* | *91%* | 100% |
| | | *Trichostrongylus* | 12% | |
| | Levamisole | *Oesophagostomum* | 0% | 0% |
| | | *Haemonchus* | 88% | 96% |
| | | *Trichostrongylus* | 12% | 4% |
| | Ivermectin | *Oesophagostomum* | 0% | 0% |
| | | *Haemonchus* | 88% | 88% |
| | | *Trichostrongylus* | 12% | 12% |
| 8 | Control | *Oesophagostomum* | 0% | 0% |
| | | *Haemonchus* | 91% | 100% |
| | | *Trichostrongylus* | 4% | 0% |
| | Albendazole | *Oesophagostomum* | 5% | 0% |
| | | *Haemonchus* | 91% | 100% |
| | | *Trichostrongylus* | 4% | 0% |
| | Levamisole | *Oesophagostomum* | 5% | 0% |
| | | *Haemonchus* | 91% | 100% |
| | | *Trichostrongylus* | 4% | 0% |
| | Ivermectin | *Oesophagostomum* | 5% | 0% |
| | | *Haemonchus* | 91% | 91% |
| | | *Trichostrongylus* | 4% | 4% |
| 9 | Control | *Oesophagostomum* | 5% | 5% |
| | | *Haemonchus* | 100% | 100% |
| | | *Trichostrongylus* | 0% | 0% |
| | Albendazole | *Oesophagostomum* | 0% | 0% |
| | | *Haemonchus* | 100% | 100% |
| | | *Trichostrongylus* | 0% | 0% |
| | Levamisole | *Oesophagostomum* | 0% | 0% |
| | | *Haemonchus* | 100% | 100% |
| | | *Trichostrongylus* | 0% | 0% |
| | Ivermectin | *Oesophagostomum* | 0% | 0% |
| | | *Haemonchus* | 100% | 100% |
| | | *Trichostrongylus* | 0% | 0% |
| 10 | Control | *Oesophagostomum* | 0% | 0% |