OCCURRENCE AND RISK FACTORS OF ENDOPARASITES AND ASSOCIATED LESIONS IN DONKEYS IN SELECTED ABATTOIRS IN KENYA

DR. NANCY NDINDA MULWA (BVM, UoN)

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DECLARATION

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

Dr. Nancy Ndinda Mulwa (BVM, UoN)

Signature: …… **Date: …….**

This thesis has been submitted with our approval as supervisors:

Signature: ……. **Date**: ……

Prof. Samuel Maina Githigia, BVM, MSc., PhD

Department of Veterinary Pathology, Microbiology and Parasitology

University of Nairobi

Signature: ……. **Date**: …….

Dr. Davis Njuguna Karanja, BVM, MSc., PhD

Department of Veterinary Pathology, Microbiology and Parasitology

University of Nairobi

Signature: ……. **Date:** ……

Dr. Cecilia Kathure Mbae, BSc, MSc., PhD

Centre of Microbiology Research

Kenya Medical Research Institute

DEDICATION

I dedicate this work to my family members for their unending love and support throughout my study period.

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LIST OF ABBREVIATIONS

<	Less than
>	Greater than
Cm	centimeters
EDTA	Ethylenediaminetetraacetic acid
EPG	Eggs per Gram
FEC	Fecal Egg Count
g	Grams
H&E-	Hematoxylin and Eosin
ID	Identity
KEMRI	Kenya Medical Research Institute
KNB	Kinamba
LDW	Lodwar
MGT	Mogotio
Mm	millimeters
MAFF	Ministry of Agriculture Fisheries and Food
MALF	Ministry of Agriculture Livestock and Fisheries
mls	millilitres
OIE	World Organization for Animal Health
PCR	Polymerase Chain Reaction
r.p.m	Revolutions per Minute
SPSS	Statistical Package for the Social Sciences
Spp	Species
UoN	University of Nairobi

ABSTRACT

The donkey is a significant animal species in the arid and semi-arid areas, it serves as an important means of transport and provision of draught power. Gastrointestinal parasites and hemoparasites have been reported to cause the most prominent diseases in donkey as compared to other diseases. Helminth infestation has been reported to cause mortality in Ethiopia besides retarding growth, decreasing work output in addition to distress and pain. Recent studies to show the diverse nature of gastrointestinal parasites and their pathological effects have not been done in Kenya. A survey was done in order to determine the diversity and intensity of donkey endoparasites and to characterize associated lesions in selected abattoirs in Kenya. Three abattoirs (Kinamba, Mogotio and Lodwar) were visited between July-September, 2017. A total of 282 donkeys presented for slaughter were systematically sampled for gastrointestinal parasites and associated lesions. Blood, fecal, parasite and gastrointestinal organ samples were collected and screened for hemoparasites, quantification of the fecal eggs, identification and characterization of pathological lesions respectively. The diverse nature of gastrointestinal parasites and hemoparasites were determined by virtue of their morphological characteristics whereas the intensity was assessed by quantifying the fecal egg count using the McMaster technique. The pathological lesions were characterized grossly and microscopically.

Adults helminths parasites identified were; *Anaplocephala magna* 2.5%, *Anaplocephala perfoliata* 10.3%, *Cylicocyclus auriculatus 2.1%, Cylicocyclus leptostomus 0.4% Cyathostome* species 2.1%, *Parascaris equorum* 20.2% , *Strongylus edentatus* 12.1%, *Strongylus equinus* 0.4%, *Strongylus vulgaris* 52.8%, *Setaria equina* 3.5% and *Triodontophorus serratus* 0.4%. Fifty one point eight percent were positive for *Gasterophilus species* larvae with an occurrence of 38.3%, 5.7% and 7.8% for *G. intestinalis, G. nasalis* and *G.pecorum* respectively. Forty four point seven percent of the donkeys were positive for

strongyle eggs; *Parascaris equorum* at 5.3%, *Oxyuris equi* at 1.1%, *Triodontophorus tenuicolis* and *Habronema* species each at 0.7% and cestodes eggs at 0.4%. For intensity determination, 55.3% had no eggs present, 39% had a low infection (up to 500 EPG), 5% had a medium infection (501-1000EPG) with 0.7% having a high infection (>1000 EPG). For the hemoparasites, *Anaplasma phagocytophilum* occurred at 2.5%, trypanosomes at 5.6%, *Babesia caballi* at 2.5% and microfilaria at 0.60% of the examined donkeys. Pathological changes observed in the stomach mucosa included diffuse areas of erosion after dislodging the various *Gasterophilus* species. Microscopic observations made included disruption of the keratinized layer of the non-glandular portion of the stomach, yellowish discoloration of the epithelium, loss of keratin and thickening in some areas. In the liver, hepatomegally was observed in 6% of the donkeys and a cyst-like focal swelling measuring 2-3 cm in diameter protruded above the capsule in 3% of the donkeys. The other 3% had pinpoint necrotic foci on the hepatic parenchyma. Microscopically, there was a well demarcated zone comprising fibrous connective tissue, hemosiderosis and cellular infiltration. In the intestines, focal nodules were present in 3% of the donkeys. Microscopic examination revealed disruption of the mucosal lining and nematodes larvae encapsulated in the sub mucosa. Intestinal ulcerations were observed in 2.5% of the donkeys and were mainly characterized by circulatory disturbances and cellular infiltrations.

The survey revealed that up to 86% of the donkeys in Kenya are infested by a variety of gastrointestinal helminths and 11.3% by hemoparasites. Gastrointestinal parasites may cause severe pathological lesions in the gastrointestinal tract and these interfere with digestion and assimilation of nutrients thereby leading to poor work performance of the donkeys. There is need to control these parasites in donkeys in Kenya.

1.0 CHAPTER ONE: INTRODUCTION

Donkeys (*Equus asinus*) are amongst the first tamed equines that have been used as draft animals for a long time (Saul *et al*., 1997). They are frequently depicted as the underprivileged man's horse, a state best revealed in the animal-energy agriculture in many of third world countries (Getachew *et al*., 2012). The global donkey population is at approximately 44 million (Starkey and Starkey, 2000). In Africa, the population of donkeys is estimated to be 13 million (Starkey and Starkey, 2001). Kenya has approximately 1,832,519 donkeys with majority of the donkeys in arid and semiarid areas (Kenya National Bureau of Statistics, 2010). Over half of this population is being used for works in transport and tillage operations (Kenya National Bureau of Statistics, 2010). The donkey has been known to play an important function in packing, riding, carting and ploughing. The main reason is due to cheapness and availability of donkeys. They are also an alternative transport means in places where the road network is inadequately developed (Pearson *et al*., 1999 and Negasa *et al*., 2017). Donkey power is an environmentally friendly means of transport and vital in areas of inadequate roads. Donkeys can easily pull more load than it can carry on condition that the harness is appropriate (Saul *et al*., 1997). Equines, mainly the donkeys have been entirely neglected regardless of their important role in both rural and urban societies (Etana *et al*., 2011)

Donkey meat is considered a delicacy in some parts of North West Kenya and Southern Ethiopia and its milk is also believed to be medicinal and has been used to treat whooping cough (Fred and Pascal, 2006). The government of Kenya recognized donkeys as a food animal in Kenya in 2012 (The Meat Control Act, 2012). A study done by Mugachia and Muthusi (2015), shows that donkey owners wish to use their donkeys for work other than for sales and that donkey owners do not uphold the consumption of donkey meat.

Currently, there are four donkey export slaughter houses in Kenya; Goldox limited, in Mogotio, Baringo County; Star-Brilliant Export slaughter House in Naivasha, Nakuru County, Silzha Limited in Lodwar, Turkana County and Fuhai Machakos Trading Company Limited. However, this venture has faced lots of resistance from local communities who do not accept or uphold consumption of donkey meat and also due to environmental degradation. In addition to this there has been a lot of donkey theft, illegal sale of the skin and meat. Due to lack of proper breeding practices, the population has been alarmingly declining. Recently there has been a report by the animal welfare lobbies that the donkeys in Kenya could be extinct in four years unless measures are put in place to guarantee the welfare of the animals. The trade in donkey meat and skin should be halted until measures are put in place to protect the species (Okech and Nyoike, 2019). The Chinese believe that donkey skin derived medicine called ejiao treats anemia, delays aging, increases libido, reverses infertility, prevents miscarriage and menstrual irregularity and treats side effects of chemotherapy (Daily Nation, 25th July 2019)

Gastrointestinal parasites and hemoparasites have been reported to be most prominent diseases in the donkey as compared to other diseases (Ahmed *et al*., 2008). Saul *et al.* (1997) reported helminth infestation as the most common cause of death besides retarding growth, decreasing work output in addition to distress and pain (Svendsen, 1997). More recently, a study in Ethiopia reported a high prevalence of gastrointestinal helminths interfering with donkey health and welfare (Mohamee *et al*., 2017). Similar studies (Lewa *et al*., 2000 and Karanja *et al*., 1994) have shown gastrointestinal parasites as prevalent in donkeys. However, there are no recent studies in donkeys in Kenya to demonstrate that parasites are still a threat to donkey welfare as well as to public health. Additionally, there is need to document the impact of these parasites and pathological lesions caused by gastrointestinal parasites in donkeys.

This research study hence aims to determine the diversity, intensity and the lesions associated with endoparasitism. This will help in planning and implementation of parasite control measures in donkeys and contribute to the improvement of donkey health and productivity in Kenya. Given that the donkey is now considered food animal, information on these parasites and subsequent control strategies will ensure farmers earn a better income for improved livelihoods.

1.1 OBJECTIVES OF THE STUDY

1.1.1 General Objective

Determine the occurrence and risk factors of gastrointestinal infestation and haemoparasitic infection and associated lesions in donkeys in selected abattoirs in Kenya

1.1.2 Specific Objectives

1. To determine the prevalence, intensity and risk factors for gastrointestinal infestation in donkeys in three abattoirs in Kenya

2. To determine the occurrence of hemoparasites in donkeys in three abattoirs in Kenya

3. To characterize lesions associated with gastrointestinal parasites in donkeys in three abattoirs in Kenya

1.2 JUSTIFICATION

Agriculture accounts for 27% of Gross Domestic Product and employs about 75% of the Kenyan population, majority of these are small holder farmers with limited finances and access to farm machinery (MALF, 2015). Consequently, agriculture is viewed as unrewarding hard job and youth are not ready for it. In addition, there is a yawning gap in food production to feed the rapidly growing human population using agricultural technology. There is need to explore draught power from donkeys and other farm animals to increase

efficiency and productivity. In Kenya's vision 2030, and Jubilee's government 2018-2022 strategic plan, food security is a priority agenda. The government is undertaking bold measures aimed at increasing acreage under cultivation in order to enhance grain supplies from arid and semi-arid lands (ASAL) through irrigation. However, there is need to match these efforts with mechanization

Donkeys are important working animals in Kenya especially the arid and semi-arid areas where people live below the poverty line. The health care of these animals is neglected as well as a vast number of the owners live in the remote areas. When in good health and well harnessed, the donkey can till possibly 100 times more land than the hand-held hoe. However, donkey power is hampered by myriad problems including malnutrition, infectious diseases and more recently booming donkey trade for slaughter that is decimating population at alarming rate. Many ignorant people believe donkeys are disease free, but incidentally, they suffer from clinical and subclinical diseases. A survey of the parasites affecting the donkeys has been done previously but not extensively as the animals involved were few. This study will also add to current information on parasites present in Kenyan donkeys. Knowledge of these parasites is important as control measures can be instituted to improve the general productivity for better use of these animals. So as to come up with effective treatment and control programs, it is crucial to have data on the occurrence of the parasites as well as the lesions they cause. Control measures include regular deworming of the animals and proper manure management.

2.0 CHAPTER TWO: LITERATURE REVIEW

2.1: OVERVIEW OF DONKEY ENDOPARASITES AND ASSOCIATED LESIONS

2.1.1: Occurrence of Various Species of Donkey Endoparasites

A parasite can be defined as a smaller organism that lives on or in a host and at the expense of the host. Endoparasites on the other hand can be defined as parasites within the body of the host (Bowman, 2014).The major categories include *Gasterophilus* species instars, *Habronema, Draschia, Trichostrongylus, Parascaris equorum, Strongyloides westeri, Anaplocephala* species, *Oxyuris equi, Onchocerca cervicalis, Setaria equina, Thelazia lachrimalis, Strongylus species* and small strongyles (Powell and Russell, 2012).

Donkeys are described as sturdy though they are vulnerable to a number of disease conditions. Among these, parasitic infections are major cause of illness in donkeys (Negasa *et al*., 2017). The deficiencies of appropriate management and by virtue of their wandering behavior, the donkeys are exposed to a wide range of parasitic infections especially gastrointestinal nematodes because they frequently come into contact with contaminated pasture (Raman *et al*., 2014). Parasites are a major problem of donkeys amid other problems such as wounds, other infectious and noninfectious diseases (Abebew *et al*., 2011).

In a research carried out in Ethiopia by Ahmed *et al* (2011), the percentage of helminth infection in donkeys was 98.45%. Twenty two (22) helminth species including *Fasciola hepatica, Anaplocephala perfoliata, Parascaris equorum,* large strongylid, small strongylids, *Habronema muscae, Habronema megastoma, Habronema microstoma, Setaria equina* and *Oxyuris equi* were identified. Hydatid cysts were also encountered. Fecal egg examination showed 99% strongyle spp, 80% *Fasciola spp,* 51% *Parascaris spp,* 30% *Gastrodiscus spp,* 11% *Strongyloides westeri,* 8% *Anaplocephala perfoliata* cestodes and 2% *Oxyuris equi*

infection prevalence. More than 55% of donkeys had higher than 1000 eggs per gram of faeces (EPG) (Ahmed *et al*., 2011).

In Ethiopia, forty two diverse species of parasites that include 33 nematodes, 3 trematodes, 3 cestodes and 3 arthropod larvae were identified from donkeys at postmortem. This study showed that working donkeys in Ethiopia harbor a variety of helminths and arthropod larvae which represent most of the significant pathogenic parasites found in equines globally (Getachew *et al*., 2010).

Thirty adult donkeys necropsied from Burkina Faso indicated that strongylids were the most common species with 100% prevalence linked to *Strongylus vulgaris*. Four species of *Strongylus*, two of *Triodontophorus* and six of the *Cyathostominae* were also identified. All donkeys were also infected with Habronematid nematodes with the oxyurid and ascaridid nematodes being less. Coprological exam of 131 samples from donkeys indicated an egg count of 100-9,200 for strongylid eggs (Vercruysse *et al.,* 1986). In a study carried out by Ibrahim *et al* (2011) in Southern Ethiopia the prevalence of helminth eggs and helminth species was 96.9%. The prevalence of gastrointestinal parasite in different age group indicated that there was a significant difference ($p<0.05$) between the young and the adult (Wako *et al*., 2016). In a current research done in Ethiopia from December 2015 to May 2016, the gastrointestinal helminths recorded by Abdulahi *et al.* (2017) were *strongyles* (79.7 %), *Parascaris equorum* (44.8%), *Oxyuris equi* (37.5%), *Fasciola* spp (1.6%) and 46.9% mixed infections. Death in equines has been commonly reported due to strongyles, tapeworms, ascarids, trypanosomes and *Babesia* species (Soulsby, 1982)

In six donkeys examined at necropsy in Kenya, the variety of species of internal parasites identified included *Dictyocaulus arnfieldi, Gastrophilus intestinalis, Strongylus vulgaris* and *Strongylus edentatus* (*Lewa et al.,* 2001). In another study done in Kenya by Karanja *et al. (*1994), the common parasites found in eight donkeys included *Gasterophilus nasalis,*

Cyathostomum tetracanthum, Habronema muscae, Strongylus spp, *Setaria equina* and *Oxyuris equi*. The fecal egg count indicated a low to moderate strongyle infestation (Karanja *et al.,* 1994). A reduction in worm burden has also been shown to result to a 14% improvement of body condition (Svendsen, 1986).

Parasites affecting the equine stomach include Gasterophilus spp. larvae and gastric nematodes. Four species of nematodes identified in the stomach include *Habronema muscae, Habronema majus, Draschia megastoma* and *Trichostrongylus axei* (Jacobs, 1986). A retrospective study done to identify causes of rectal prolapse in donkeys showed that majority of rectal prolapse cases were linked to *Gasterophilus nasalis*. Other causes included overworking the donkeys and diarrhea (Getachew *et al*., 2012).

Genus echinococcus has two important species of veterinary significance and these include *Echinococcus granulosus* and *Echinococcus multilocularis*. *Echinococcus granulosus* equinus is the sub species whose larval stages (hydatid cysts) are found in the equines. A case of hydatid cyst (*E.granulosus*) in the liver of a horse was demonstrated with the horse displaying no clinical signs despite the presence of 20-30 cysts (Bowman, 2014). Evidence of hydatidosis was established at 17.2% of 122 donkeys necropsied in Central Jordan (Abo-Shehada., 1988).

Equine has been reported as a less common host for Fasciola and the rate of infection is relatively low as compared to the ruminants (Jones et al., 1977). Necropsy of 65 donkeys in Egypt revealed that only two donkeys were infested with *Fasciola hepatica* in their bile ducts indicating a low occurrence (Ahmed *et al*., 2011).

Microfilaria was diagnosed in 20 blood samples collected from healthy horses with a prevalence of 30.76% (Suleiman *et al*., 2012). *Setaria equina* could affect the donkey eye

with severe lesions such as continuous lacrimation and ulcerative dermatitis (Suleiman *et al*., 2012).

Sharma and Pachauri (1981) showed that the filarial worms meet their nutritional requirement from the host tissues and fluids and in situ transfer of nutrients from the host tissues to the body of microfilariae and this causes various deficiency symptoms including anemia depending on the worm load of the host. In a study undertaken in Hungary, 9.2% of the donkeys were infected with *Setaria equina* (Hornok *et al*., 2007).

2.1.2: Lesions Associated with Nematodes

Cyathostomes result in an inflammatory enteropathy affecting the caecum and colon (Love *et al*., 1999). When the cyathostomes move to the surface from the bowel, they cause rupture of the muscularis mucosae, local eosinophilia, edema and infiltration by neutrophils and macrophages (Maxie *et al.,* 2016).

Hepatic macroscopic changes associated with experimental infection with *Parascaris equorum* infection included focal hemorrhages and tiny, white diffuse or nodular lesions. Microscopically, lesions were seen mainly around the portal triads and were characterized by cellular infiltration of eosinophils and lymphocytes as well as fibrosis (Brown and Clayton, 1979).

Trichostrongylus axei has been associated with chronic catarrhal gastritis, which may lead to weight loss. The lesions include nodular areas of thickened mucosa bordered by a zone of congestion and covered with an inconsistent amount of mucus (MSD Veterinary Manual, 2016). The third stage larvae of *Trichostrongylus axei* enters the tunnels in the epithelium of the gastric glands in the fundic and pyloric regions leading to mucous metaplasia and hyperplasia of the glands. Cellular infiltration by eosinophils and lymphocytes will be seen in the lamina propria (Maxie *et al.,* 2016)

In the livers of the donkeys, traumatic hepatitis, bile duct thickening and abscess formation were seen and were linked to *Strongylus* species larvae. Sections of parasitic larvae were observed to be connected with hemorrhage and cellular degeneration. The liver tissue was also highly infiltrated with eosinophils (Lewa *et al*., 2000).

Both the large and small strongyles have been shown to cause enteritis with the mucosal surface having parasitic nodules. The intestinal mucosa in the majority of the donkeys was highly folded and covered with mucoid material. Microscopically, the mucosa and the sub mucosa had cellular infiltrations (Lewa *et al*., 2000). *Hemomelasma malei* are subserosal hemmorhagic lesions associated with the migration of strongyle larvae (Maxie *et al.,* 2016). *Strongylus vulgaris* has been linked to endo-arteritis and thrombi formation. *Strongylus equinus* has been associated with formation of cecal and colonic nodules as they penetrate the mucosa and hemmorhagic tracts in the liver and pancreas (Soulsby, 1982). The nodules are surrounded by debri, polymorphonuclear cells and macrophages (Maxie *et al.,* 2016). *Strongylus edentatus* on the other hand has been associated with hepatic hemorrhagic nodules in the liver associated with the 4^{th} stage larvae 3-5 months post infection (Soulsby; 1982). Parenchymal scars and tags of fibrous tissue on the liver capsule have also been associated with *Strongylus edentatus* (Maxie *et al.,* 2016). *Strongylus equinus* has been associated with hemorrhagic subserosal nodules in the caecum and colon (Maxie *et al.,* 2016)

Invasion by *Habronema* species triggers the secretion of great quantities of thick and tenacious mucus in the glandular portion of the stomach adjacent to *Margo plicatus,* where adult worms are implanted (Jacobs, 1986). Gastric habronemiasis is accompanied by catarrhal gastritis, diarrhea, progressive weight loss and ulcers (Traversa *et al*., 2006). Histopathological survey indicated two distinct lesions characterized by widespread infiltration of inflammatory mononuclear cells with some eosinophils in the mucosa and submucosa. A huge granulation mass located in the deep layer of the submucosa containing

granulomatous necrotic tissue and cross sections of Dras*chia megastoma* were also seen (Nadalian *et al.,* 1997). Grossly, *Draschia megastoma* causes tumor-like nodules in the *Margo plicatus* when the parasites burrow in the sub-mucosa (Maxie *et al.,* 2016). *Triodontophorus* and *esophagodontus* species are linked with nodules in the mucosa and sub-mucosa of the caecum and colon (Maxie *et al.,* 2016).

2.1.3: Lesions Associated with *Gasterophilus* species

In a study done by Al-Mokaddem *et al* (2015) in Egypt, *Gasterophilus* larvae infestations were discovered in every case where the larvae were attached to the non-glandular part of the stomach, gastric outlet and the proximal duodenum. Upon removal of the larvae, tiny superficial erosions were seen at their sites of attachment. Microscopically, the attachment site appeared as a deep, pitted ulcer with exposed lamina propria, granulation tissue formation and neutrophilic infiltration. Grossly, the stomach mucosa revealed hyperemia, edema, erosions and ulcers. Microscopical examination showed hyperkeratosis, acanthosis, vacuolar degeneration of stratified squamous cells, gastritis, erosions, ulcerations, scarring, hyperactivity of mucus glands, periglandular fibroplasia and parasitic granulomas with infestation by *Gasterophilus* larvae (Al-Mokaddem *et al*., 2015). The epithelial margins and deep layers of the stomach also develop rete pegs (Maxie *et al.,* 2016). Additional health concerns that may result due to high infestation of these larvae are; chronic gastritis, gastric ulcers, esophageal paralysis, peritonitis, rupture of the stomach, squamous cell tumors and anemia (Williams and Knapp, 1999).

2.1.4: Lesions Associated with Cestodes

Macroscopic and microscopic lesions linked to *Anaplocephala perfoliata* include mucosal erosion, ulcerations, edema accompanied by eosinophilic and mononuclear infiltration in the mucosa and basal membrane of the caecum and colon (Sangioni *et al*., 2000).

The tapeworm larvae (Hydatid cyst) form large cysts in the liver of the donkey which can occlude the liver (Svendsen, 1986).

2.1.5: Lesions Associated with Trematodes

Hepatic fibrosing granulomas were found to be a result of chronic schistomiasis due to the eggs that were escaping from the blood vessels containing the adults (Buergelt and Greiner., 1995).

The liver may have an irregular outline and appear pale and firm. In chronic cases, hepatic fibrosis and hyperplastic cholangitis is usually seen. The types of fibrosis include post necrotic scarring, ischaemic fibrosis and fibrosis associated with flukes in the bile ducts. Calcification of the bile ducts is more common in bovines (Boray and Murray., 1999). In Egypt, bovine histopathological examination of tissues due to acute hepatic fascioliasis showed hepatic necrosis and degeneration with numerous abscesses in the hepatic parenchyma that was characterized by homogenous mass of necrotic cells bordered by heavy aggregations of inflammatory cells chiefly neutrophils, histiocytes and lymphocytes. The abscesses were surrounded by fibrous connective tissue capsule (Sohair and Eman, 2009). Chronically, increased fibrous connective tissue in the portal triads was seen. The biliary epithelium were hyperplastic characterized by formation of a large numbers of new bile ductules and existence of mature fasciola worm inside the lumen of the main bile ducts(Sohair and Eman, 2009).

2.2: Animal Risk Factors for Occurrence of Gastrointestinal Parasites

Significantly higher positivity for helminths has been observed in males (85.18 %) when compared with female (50%) donkeys (Sathiyamoorthy *et al*., 2016). According to Jajere *et al* (2016), age, sex and season were not statistically associated with the risk of helminth infection. On the other hand, body condition scores, settlement, anthelmintic medication history and management practices were significantly linked with the risk of gastrointestinal helminthosis. A high prevalence of helminthic infections was seen in donkeys that had a poor body condition, those from rural settlements, those with no anthelmintic treatment and those that are raised under poor management systems (Jajere *et al*., 2016). In a study done in Ethiopia by Ibrahim *et al* (2011) they established that body condition was an important factor that influenced the occurrence of some helminth parasites as the parasites were more prevalent in animals with a poor body condition than those that were well conditioned. It was then recommended that donkey owners ought to be trained to better the management system, particularly with regard to the level of nutrition to ensure that the donkey has a good body condition that bestows some level of resistance against helminth infection (Ibrahim *et al.*, 2011)

2.3: Occurrence of Donkey Hemoparasites and Associated Lesions

African animal trypanosomiasis is one of the key hurdles to livestock development and agricultural production (Mekuria *et al*., 2010, Boada-Sucre *et al*., 2016). In a cross sectional study of trypanosomosis done in South Ethiopia, 10.7% of the donkeys were positive for *Trypanosoma vivax* and *Trypanosoma congolense* as seen by dark ground and phase contrast buffy coat method and Giemsa stained blood smears (Mekuria *et al*., 2010).

Three species of trypanosomes identified in donkeys by Abebe and Wolde (2010) included *Trypanosoma congolense* (52.4%), *Trypanosoma brucei* (28.6%) and *Trypanosoma vivax*

(19.05%). The observed red blood cell alterations are as a result of mechanical and biochemical damage due to host-parasite interaction that occurs in the bloodstream (Boada-Sucre *et al*., 2016). Additionally, the morphological changes of the red blood cells in *Trypanosoma vivax* infection are seen to be a contributory factor for the pathophysiology of the disease (Boada-Sucre *et al*., 2016). In an experimental infection carried out in five donkeys in Brazil, *Trypanosoma evansi* infection resulted to decrease in hemoglobin levels, packed cell volume and erythrocyte count. Biochemically, the icteric index and serum globulins was high. The serum albumin and glucose levels had also decreased. At necropsy the donkeys exhibited splenomegally characterized by increased white pulp, lymphadenopathy of the mediastinal lymph nodes, pulmonary and hepatic congestion as well. Meningoencephalitis was also seen and was characterized by perivascular cuffing. Demyelination in some aspects of the cerebellum pediculus and neutrophil vacuolization was observed. Hemosiderosis and lymphoid hyperplasia in the lymph nodes and spleen was seen. The kidneys also exhibited chronic nephritis (*Cadioli et al*., 2006). In a more recent study undertaken by Garba *et al* (2016) in Nigeria, a gross and histopathological study was done on 18 donkeys, where six of them were infected with *Trypanosoma spp*. The gross lesions in six donkeys included lung congestion with frothy exudate in the trachea in cases that were acute. In chronic cases, grey hepatization, hydrothorax, hydroperitoneum, serous atrophy of fat and mild adherence of renal capsule was observed. Microscopically, the lungs showed congestion and mononuclear cellular infiltration. Hemosiderosis and lymphocytic depletion was seen in the germinal centres of the spleen. The liver was congested with moderate focal necrosis and cellular infiltration of mononuclear cells in nature (Garba *et al*., 2016).

Poikilocytosis is a general terminology that refers to red blood cells with an abnormal shape. The specific cell changes can also be described and are of diagnostic significance. Acanthocytes are defined as sphere shaped red blood cells with blunt tipped spicules that are

irregular in pattern at the margins. Echinocytes/crenated red blood cells are those that have sharp blunt spicules of uniform length and are more evenly spread at the periphery. In horses, the echinocytes may have blunt ends owing to relatively smaller sizes of red blood cells. This morphological alteration has been seen in horses with colic and diarrhea (http://eclinpath.com/hematology/morphologic-features/red-blood-cells/poikilocytosis/).Light microscopic studies have demonstrated that *Trypanosoma evansi* produces poikilocytosis (Silva *et al*., 1995).

Morphological alterations in the ovine red blood cells due to *Trypanosoma evansi* as observed under scanning electron microscope in Venezuela included vesicle formation in the red blood cell, microspherocytosis and deformation of the red blood cells (Boada-Sucre *et al*., 2016). Several erythrocytes abnormalities were observed in the blood of *Trypanosoma* spp infected dogs and horses and included the appearance of microspherocytes, acanthocytes, dacrocytes, codocytes, vacuolated and sometimes bizarre shapes of red blood cells. Polychromasia and poikilocytosis were present in both dogs and horse erythrocytes (Silva *et al*., 1995).

Equine piroplasmosis is a tick-borne disease caused by hemoparasites namely; *Babesia caballi* and *Theileria equi* (Formerly *Babesia equi).* It is a notifiable disease and a key limitation to the international movement of equines (Gizachew *et al*., 2013). *Babesia equi* has however been moved to the genus *Theileria* by Mehlhorn and Schein based on its preerythrocytic developmental stages in the lymphocytes (Mehlhorn and Schein, 1998). Infected animals may remain carriers for protracted periods and act as sources of infection for ticks, which are the vectors (OIE, 2014). In acute clinical cases when the parasitaemia is high it is difficult to detect the parasite and for this reason serological methods are preferred though they may also give a false positive/negative (OIE, 2014). Necropsy findings include; anemic and icteric organs, hydrothorax, ascites, hydropericardium, subcutaneous edema,

splenomegally, hepatomegally with the distension of the gall bladder, pulmonary congestion and lymphadenopathy. In cases of *Theileria equi*, mucosal hemmorhages and hypertrophy with accompanying inflammation of the lymph nodes is more pronounced (Brandt, 2009). In a retrospective study done by Rassouli and Aghazaman (2015), a certain group of dog blood films were subjected to examination to look for tick borne pathogens and the red blood cells showed polychromatophilic macrocytes, hypochromic reticulocytes, poikilocytosis and anisocytosis. Sex-related variation in the prevalence of Theileria and Babesia infections in donkeys is absent as shown by serohematological study (Gizachew *et al.,* 2013).

Babesia caballi and *Theileria equi* are most often associated as they share common vectors. However, infections with *Theileria equi* are more prevalent than *Babesia caballi* infections (Friedhoff *et al*., 1990).

Setaria equina, family: *Onchocercidae*, subfamily: *Setariinae*; is a nematode filarial parasite usually found in the peritoneal cavity of equines in diverse geographical regions of the world (Coleman *et al.,* 1985). The adult; in the peritoneal cavity is usually harmless and are found incidentally during pathological examination (Hillyer *et al*., 2001). The infection is transmitted by mosquitoes. The sheathed microfilaria worms are found in the blood (Yeargan *et al.,* 2009). Microfilaria of equine in Baghdad was reported to have an occurrence rate of 11.11%. The technique used for screening was Knott technique (Afkar and Amall, 2014). Another study in Hungary reported a percentage occurrence of 9.2 of microfilaria of *Setaria* spp using a similar technique which is regarded as a more sensitive (Hornok *et al.,* 2007). In Iraq, the percentage of infection amongst horses was 30.76% by Knott technique (Suleiman *et al*., 2012). The presence of adult worms in the peritoneal cavity is not always accompanied by microfilaraemia (Hornok *et al.,* 2007).

3.0 CHAPTER THREE: MATERIALS AND METHODS

3.1: Study Areas

Three study sites were purposively selected (**Figure 1**) based on the presence of donkey abattoirs. These included:

Site A: Silzha Company in Lodwar town, Turkana County. Turkana County is the second largest in terms of land area. It is bordered by the country of Uganda to the west. Lodwar is the largest town and is Turkanas' capital (Wikipedia, 2019).

Site B: Goldox Kenya Limited in Mogotio, Baringo County. The economy is based on agricultural activities with hides and skin as one of the main livestock products. Mogotio is one of the urban centres (Wikipedia, 2019).

Site C: Star Brilliant Donkey Export Slaughter House in Naivasha, Nakuru County. Naivasha is a major significant urban centre in Nakuru County (Wikipedia, 2019).

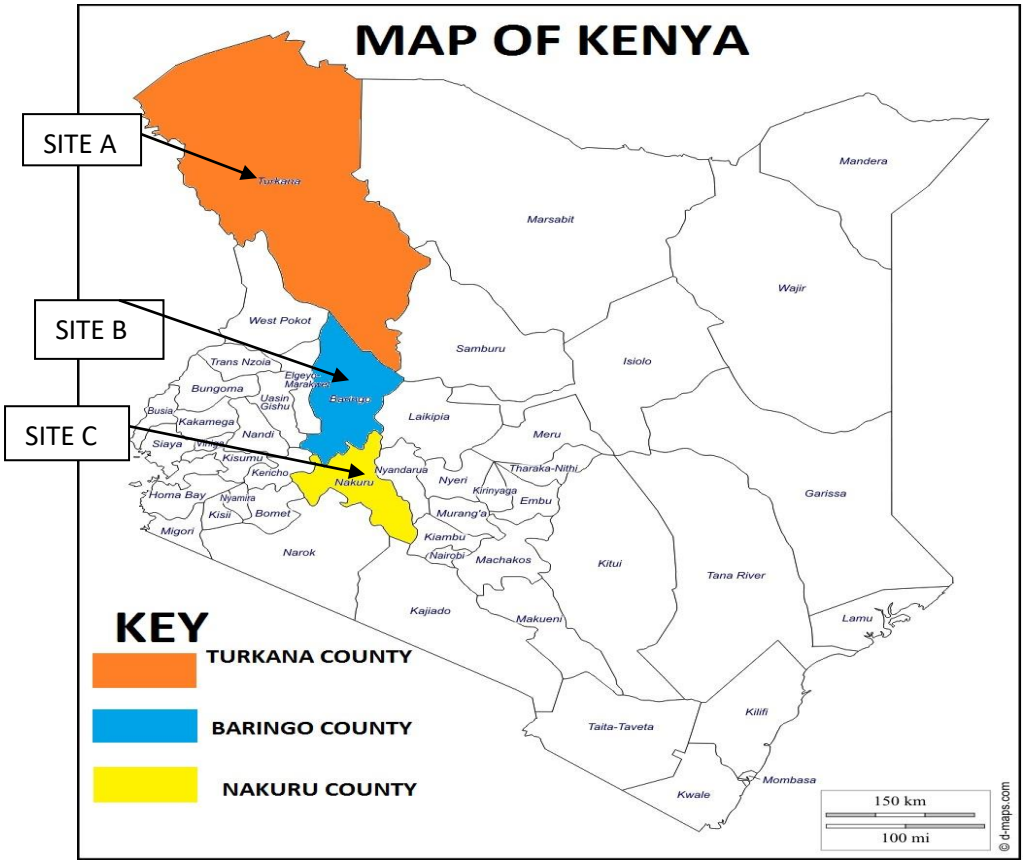

Figure 1: Map of Kenya showing the three Sites of Study; Turkana (A), Baringo (B) and Nakuru (C) County (Source: d-maps.com accessed at https://d-maps.com/pays.php?num_pay=30&lang=en)

3.2: Study design

A cross-sectional study was undertaken in the three donkey slaughter houses in the month of July to September 2017 in order to examine the donkeys, collect blood, fecal, parasite and organ samples for hemoparasites, parasitological and pathological analyses respectively.

Upon arrival at the slaughter house, the movement permits of the donkeys were verified by the meat inspector. The animals were then subjected to antemortem inspection and put in holding pens with water *ad lib*. The period the animals were put in the holding pens depended on the total number of donkeys present for slaughter. If the donkeys present were small in number, they weren't slaughtered due to economic reasons. The donkeys for slaughter were

led into the lairage and then into the stunning box where they were humanely killed via use of a captive bolt piston. They were then hoisted up and the jugular vein slithered to achieve bleeding. Skinning was then done followed by evisceration of gastrointestinal tract and the red offals. Each section of the gastrointestinal system was then inspected.

3.3: Sample Size Determination

The sample size was determined according to the number of animals presented for slaughter. A total of 282 out of the 1,538 donkeys presented for slaughter were examined for gastrointestinal parasites. Systematic sampling method was applied at the slaughter house level whereby every 5^{th} animal was examined depending on the number presented for slaughter. While examining the thin blood and buffy coat smears simple random sampling method was used and the random numbers for the slides to be examined was obtained via use of a scientific calculator (FX-82 MS). A total of 160 thin blood smears and 160 buffy coat smears were examined under light microscope. The formulae used to derive the sample size for the smear examination was as follows:

In a finite population; N=282

n= 384 which is the sample size for an infinite population

Adjustment; where n (the new sample size);

$$n = 1/(1/n + 1/N)$$

n=1/ (1/384+1/282) = 162 (Hewson, 2004).

Sampling proportional to size:

Mogotio; 114= 114/282=0.4X 162=65

Kinamba; 148= 148/282= 0.52X162=84

Lodwar; 20= 20/282=0.07X162=11

0.4+0.52+0.07=0.99≈ 1

All samples=160X 2=320 thin blood and buffy coat smears.

65+84+11=160

3.4: Determination of Prevalence and Intensity of Gastrointestinal Parasites and Risk Factors for Helminth Infestation in Donkeys

3.4.1: Prevalence of Gastrointestinal Parasites

Collection of Gastrointestinal Parasites and Identification: The gastrointestinal system was examined from the esophagus to the rectum and parasites were picked from the gastrointestinal tract and the liver. The gross lesions linked with the parasites were noted down. The parasites were then rinsed gently with water and preserved in 80% alcohol for further analysis. The parasites were then identified based on their gross/microscopic morphological features as described by Soulsby (1982). Cestode samples collected were processed according to a technique described by International Institute of Parasitology (1994) and identified morphologically according to Soulsby (1982). The arthropod larvae were obtained from the gastric mucosa by use of a forceps. The different species were identified by virtue of their morphological features as described by Soulsby (1982). The proportion of donkeys positive for a specific parasite was determined in terms of percentages.

3.4.2: Intensity of Gastrointestinal Helminths Infection

Fecal sample (5-10 grams) was collected directly from the rectum of each animal post slaughter so at to determine the intensity of helminth infection. The sample was labelled and preserved in 10-15 mls of 80% alcohol to prevent hatching of the eggs. The samples were transported to the Parasitology laboratory at the department of Veterinary Pathology, Microbiology and Parasitology (UoN). McMaster egg count technique was applied in the processing of the samples according to MAFF (1986). Identification of the helminth eggs and

cysts was done and their counts performed. The intensity of infection for strongyle eggs was recorded for each donkey and classified according to established infection intensity classes into one of the four classes: none, low (up to 500EPG), medium (501-1000 EPG), high (>1000 EPG) (Soulsby 1982).

3.4.3: Risk Factors for Helminth Infestation in Donkeys

Data regarding approximate age, sex, origin of the animal, pregnancy status, physical strength and the body condition score estimate were noted (Appendix 1.0). The body condition scores were given in a scale of 1 to 5(1-Poor 2-Moderate, 3-Ideal 4-Fat 5-Obese) according to National Equine Welfare Council (2005); (see attached appendix 2). This data was compared with the fecal strongyle eggs intensity and the prevalence of the adult helminths.

3.5: Determination of Occurrence of Hemoparasites in Donkeys

To determine the proportion of donkeys infected with hemoparasites, the number of donkeys positive for hemoparasite was divided by the overall number of donkeys examined and multiplied by 100.

$$\text{Occurence} = \frac{\text{Total no. of donkeys positive for a specific hemoparasite}}{\text{Total number of donkeys examined}} \times 100$$

Sample collection and processing: A 10ml venous blood sample was collected from each animal during bleeding after the donkey was stunned. A thin blood smear was made immediately, air dried and fixed in absolute methanol. The remaining volume of the blood sample was immediately transferred into an EDTA blood vacutainer and the whole lot was centrifuged at 300 r.p.m for 15 minutes to separate blood components. A thin buffy coat smear was prepared, fixed in absolute methanol and stained using Giemsa (1:5dilution). The smear was then screened for the presence of hemoparasites under the oil immersion objective

lens (×1000). Identification of hemoparasites was based on their morphological features (Brar *et al.,* 2002). The cellular changes on thin blood smears were also noted down.

3.6 :Characterization of Lesions Associated with Gastrointestinal Parasites in Donkeys in Selected Abattoirs in Kenya

The postmortem inspection of gastrointestinal tract was carried out in the green area whereas the liver was inspected in the red area. The green and red area areas are specific zones in the slaughter where the inspection of these organs is carried out. The lesions associated with helminths were characterized using the macroscopic and microscopic appearance. Gross lesions were identified and characterized according to their variation in size, shape, color and consistency as compared to normal organs. Special attention was paid to liver, stomach and the intestines. This was because; a previous pilot study done by Mulwa *et al* (unpublished data) that indicated that the helminth infestation was high in these organs. Gastrointestinal organ samples were collected and fixed in 10% buffered formalin then processed routinely via the paraffin wax method and stained with Hematoxylin and Eosin according to Carson and Hladik (2009). The tissue sections obtained thereafter were examined using a light microscope at X40, X100 and X400 magnifications. The lesions observed at each section were then recorded as per the organ affected and type of lesion.

3.7: Data Analysis

The data was entered into Microsoft Office Excel Data sheet (2007), cleaned and coded. It was later on transferred into statistical software (SPSS) version 22 for descriptive statistics and various association tests. The proportion of the donkeys with the parasites was determined using simple percentage methods. The proportion of the four classes of strongyle egg infection was also determined. Prevalence estimates were obtained for overall infection. Nonparametric correlation tests and independent t test were used to quantify associations

between independent variables (age, pregnancy status and sex) and intensity of strongyle infection as well prevalence of helminths. The level of significance was set at $p<0.05$. Results were considered significant in cases where the P value was less than 0.05. The confidence level was held at 95%.

4.0 CHAPTER FOUR: RESULTS

4.1: GENERAL INFORMATION

A total of 282 donkeys were examined; 20 from Lodwar, 114 from Mogotio and 148 from Kinamba slaughterhouses (**Table 1**). Of these, 40% (114/282) were females while 60% (168/282) were males. Ninety four percent of the sampled animals were adults whereas 6% were juveniles. The number of animals observed to be weak prior slaughter were 1.4% (4/282) of the animals while 98.6% (278/282) of the animals appeared physically strong.

A high proportion of the animals had a poor body condition (89%; 250/282). Moderate body condition was recorded in 11% (32/282) of the animals. Samples examined included; 36 tissues from the gastrointestinal system, 282 fecal, 160 blood smears and 160 buffy coat smears.

Table 1: Proportion of Animals Examined per Slaughterhouse

	Lodwar	**Kinamba**	**Mogotio**	**Total**
No. slaughtered	70	148	1320	1538
No. sampled	20	148	114	282
Proportion of the sampled animals	(20/282); 7.1%	(148/282); 52.5%	(114/282); 40.4%	100%

Gravid females donkeys presented for slaughter were 16.7% (19/114) whereas non-gravid ones were 83.3% (95/114). Out of the 19 gravid female donkeys, 52.6% (10/19) were positive for strongyle eggs whereas, 47.4% (9/19) females were negative for strongyle eggs. The

study found out that pregnant females had no statistically significant fecal egg count (178 ±265.8 E.P.G) as compared to the non-pregnant females (141.1 ±228.5 E.P.G)

(P=0.522) (**Table 2**).

Table 2: Mean Egg Count between the Gravid and Non-gravid Female Donkeys

	Pregnancy status	No. of female donkeys	Mean of the strongyle eggs	Std. Deviation	Std. Error Mean
Strongyle egg count	Pregnant	19	178.9474	265.78803	60.97596
	Not Pregnant	95	141.0526	228.54523	23.44825

4.2: Prevalence and Intensity of Gastrointestinal Parasites and Risk factors for Helminth Infestation in Donkeys in Three Abattoirs in Kenya

4.2.1: Intensity: Fecal Egg Count

Fifty five point seven percent (157/282) of the fecal samples analyzed using the McMaster technique were positive for helminth eggs while 44.3% (125/282) were negative. Fifty five point three percent of the donkeys (156/282) of the donkeys were negative for strongyle eggs, 39% (110/282) had a low infection rate (up to 500 EPG), while 5% (14/282) had a medium infection rate (501-1000EPG) with 0.7% (2/282) having a high infection rate (>1000 EPG). See table 3 below.

Table 3: Table showing the intensity of the Strongyle egg infection rates for slaughtered donkeys

Level of Infection	No. of donkeys in each category	Proportion (%)
High (>1000)	2	0.7
Medium (501-1000)	14	5
Low(up to 500)	110	39
None	156	55.3
Total	**282**	**100**

4.2.2: Animal Risk Factors and Helminth Infestation

There was no significant difference in the fecal strongyle egg count between the adults and the juveniles (P=0.283). Additionally, there was no significant difference in strongyle eggs shed between males and females (P=0.529). There was a significant difference in the strongyle egg count shed between the poor and moderate body condition scores (p= 0.001). A correlation test to determine the presence of helminth parasite in the age and sex revealed that there was no association between age and sex of a donkey and the presence of helminths (p=0.739; p=0.624). There was no association between body condition scores and the prevalence of the helminths (X^2(O.121[a], p=0.488). (Appendix 2).

4.2.3 Prevalence of the Helminth Eggs in the Slaughtered Donkeys

A total of 44.7 % (126/282) of the donkeys were positive for strongyle eggs(**Figure 3**), *Parascaris equorum* affected 5.3% (15/282) of the donkeys(**Figure 4**); followed by *Oxyuris equi* at 1.1% (3/282); **(Figure 5**),*Triodontophorus tenuicolis* and *Habronema* both at 0.7% (2/282) and cestodes eggs had the lowest prevalence at 0.4% (1/282) (**Figure 2**). The means and ranges of the various eggs and cyst were demonstrated. The strongyle egg counts ranged from 0-1900 with an average of 136.52 eggs per gram of feces (EPG) followed by *Parascaris equorum* with a mean of 13.12 EPG. The least was the cestode egg with a mean of 0.35 EPG. Coccidia and Giardia cysts had an average of 3.90 and 2.13 oocysts per gram of feces respectively (**Table 4**).

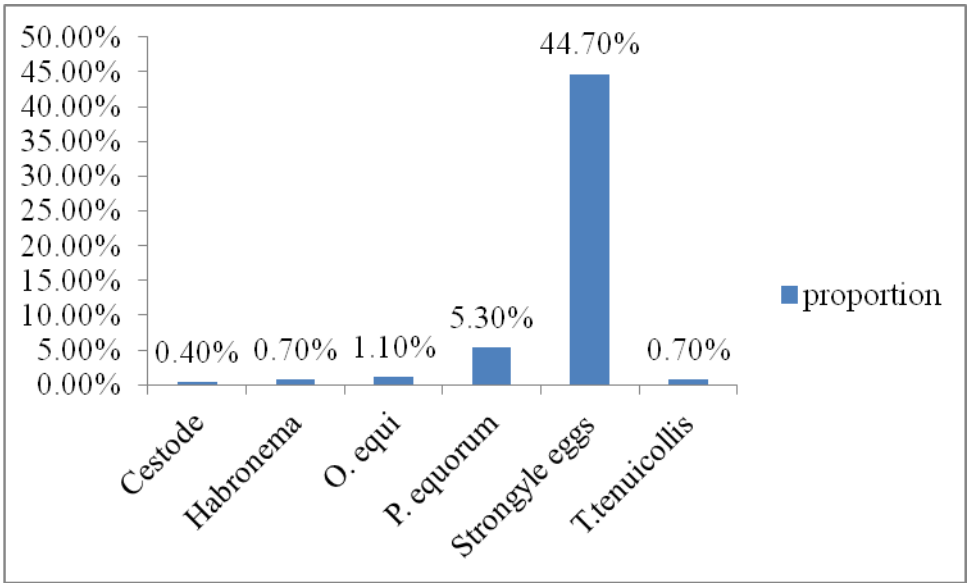

Figure 2: Prevalence of the Helminth Eggs in Fecal Samples of Slaughtered Donkeys

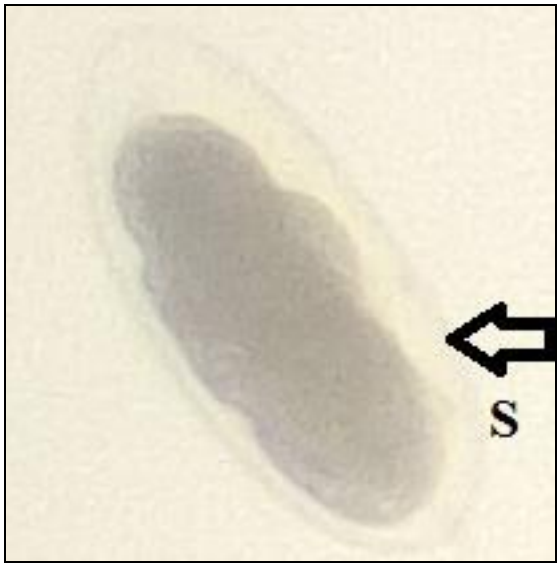

Figure 3: Photomicrograph showing a typical strongyle type egg(S), thin shelled and ovoid in shape from donkey (ID: 22 KNB)

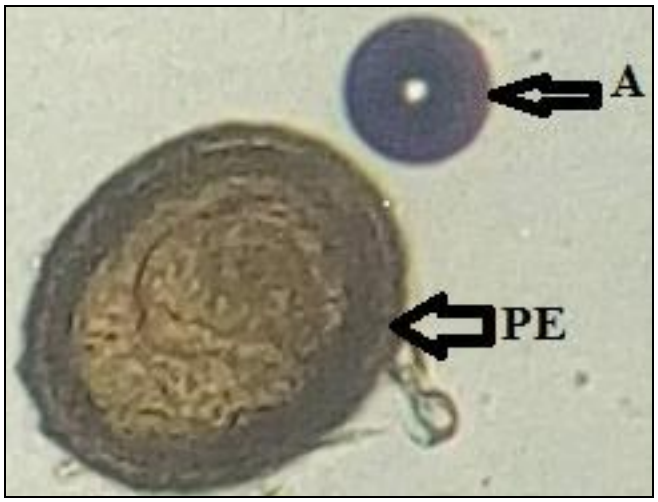

Figure 4: Photomicrograph showing the spherical brownish egg of *Parascaris equorum* (PE) from donkey (ID: 51 KNB) and an air bubble (A).

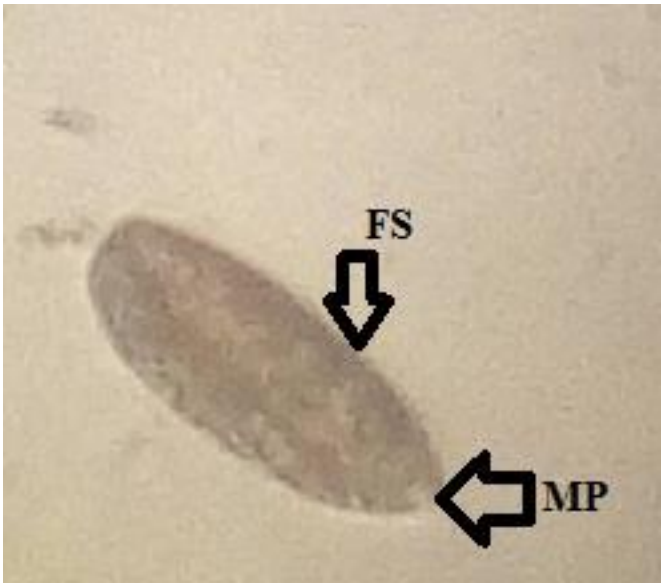

Figure 5: Photomicrograph Showing the Egg of *Oxyuris equi* from donkey (ID: 75 MGT) the mucoid plug (MP) and the flattened side (FS) are illustrated.

Table 4: Showing infestation intensity ranges and means ±standard error of the mean of helminth eggs, cysts and oocysts recovered from the fecal samples

Identity of the eggs/cysts	**Number sampled**	**Intensity ranges**	**Mean± Standard error of the mean**
Cestode spp	282	0-100	0.35±.355
Coccidia spp	282	0-500	3.90±2.027
Giardia spp	282	0-400	2.13±1.584
Habronema spp	282	0-100	0.71±.501
Oxyuris equi	282	0-300	2.13±1.323
Parascaris equorum	282	0-500	13.12±3.660
Strongyle eggs	282	0-1900	136.52±14.153
Triondontophorus tenuicolis	282	0-100	0.71±.501

4.2.4: Prevalence of the Cysts

A total of 2.1% (6/282) of the donkeys were positive for Giardia cysts while 0.7%(2/282) of the donkeys were positive for coccidian oocysts.

4.3: Occurrence of the Various helminths in Donkeys and Associated Lesions

Out of the 282 donkeys sampled, 85.5%(241/282) were positive for various helminth parasites whereas 14.5%(41/282) were negative. A total of 36 donkeys had severe gross lesions. Twenty two were from Mogotio, eleven from Kinamba and three from Lodwar slaughterhouses. Lesions were mainly found in the intestine 47.2% (17/36), followed by the liver 41.7% (15/36) and lastly the stomach at 11.1% (4/36).

4.3.1 Stomach

In the stomach, *Gasterophilus species* occurred at a rate of 51.8% (146/282). A proportion of 38.3% (108/282) of the animals were positive for *G. intestinalis*, *G.pecorum* had an occurrence of 7.8 % and 5.7% of the donkeys were positive for *G. nasalis* (**Figure 6**).

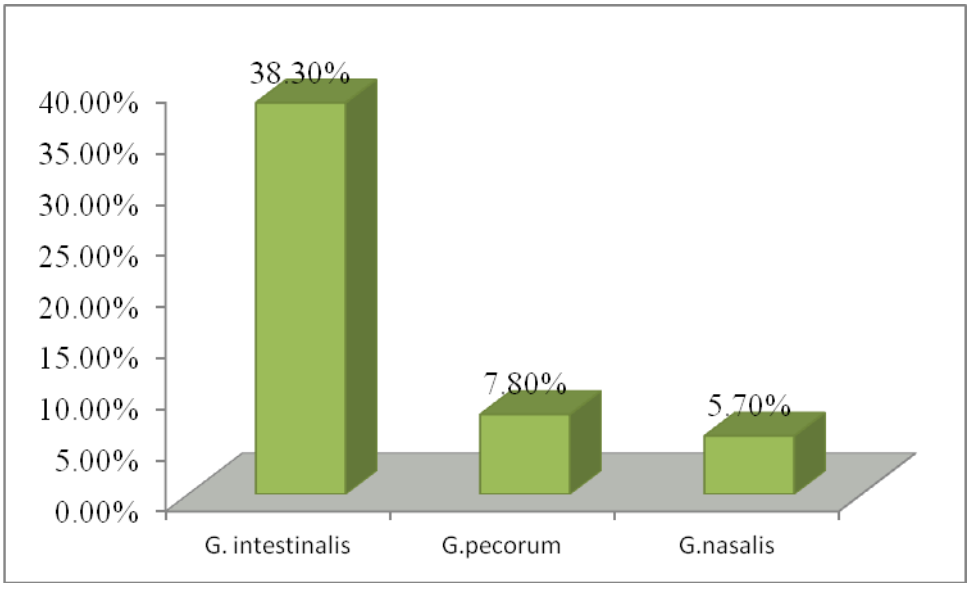

Figure 6: Proportion of the various species of the *Gastrophilus* larvae in the stomach of slaughtered donkeys

The area that was affected by *Gasterophilus* species was the non-glandular region of the stomach. Grossly multiple ulcers that were regular, circular with elevated margins and a depressed center were observed after dislodgement of the various *Gasterophilus* species. The mucosa was tinted yellow in some areas (**Figure 7**). In a donkey (ID: 72 MGT) the keratinized layer of the non-glandular epithelium of the stomach had peeled off**.** Microscopically, the mucosal epithelium was thickened in some areas and there was loss of tissue architecture with accompanying dilatation of the sub-mucosal blood vessels. Additionally there was disruption of the keratinized layer of the non-glandular stomach. The keratin covering the surface epithelium had a yellow tinge. Empty white spaces were visible in the lamina propria (**Figure 8, 9 and 10**). In another case there was a notable neutrophilic cellular infiltration in the exposed lamina propria and accompanying formation of rete pegs. Vacuolar degeneration was observed in one case and appeared like empty white spaces below the mucosa (**Figure 10**).

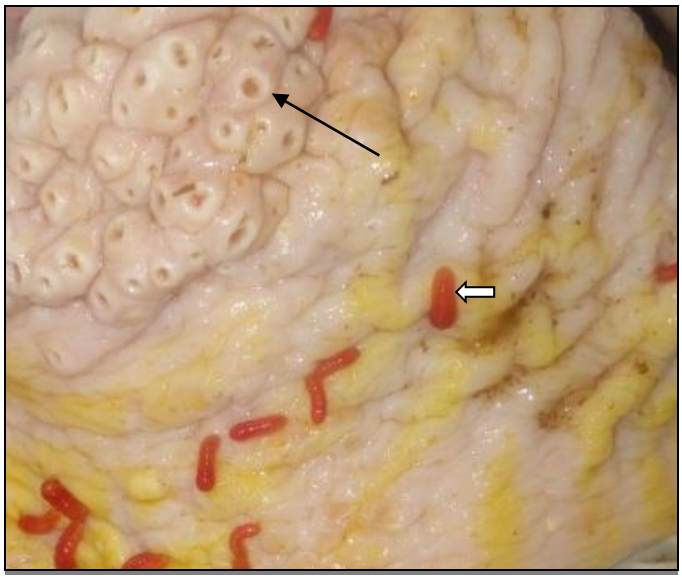

Figure 7: An ulcer (shown by the black arrow) on the non-glandular area of the donkey stomach with some areas of the mucosa tinted yellow from donkey (ID: 20 KNB); several *Gasterophilus* species are present (white block arrow).

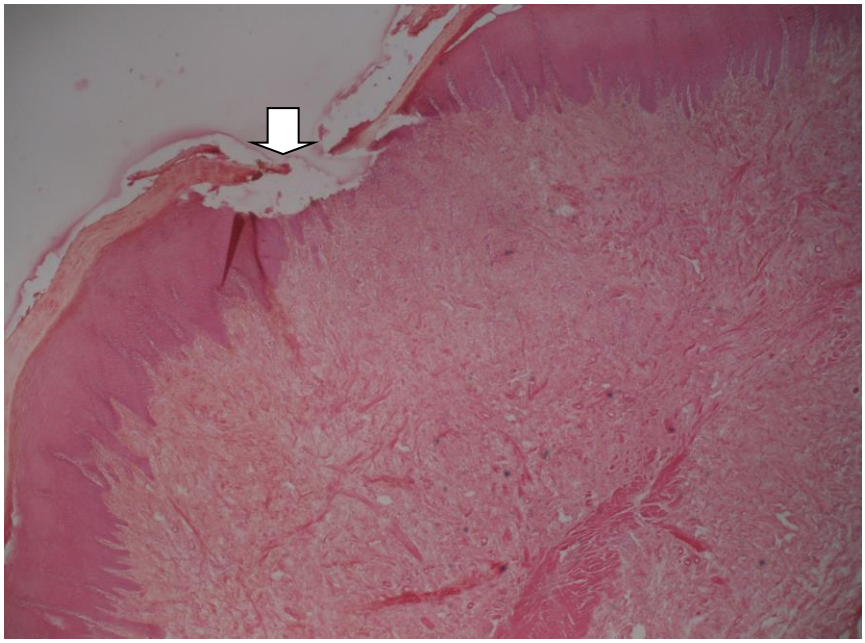

Figure 8: Photomicrograph of a stomach section of a slaughtered donkey (20 KNB) illustrating the disruption of lamina propria (white block arrow) and discontinuity of the stratified squamous epithelium (X10; H&E)

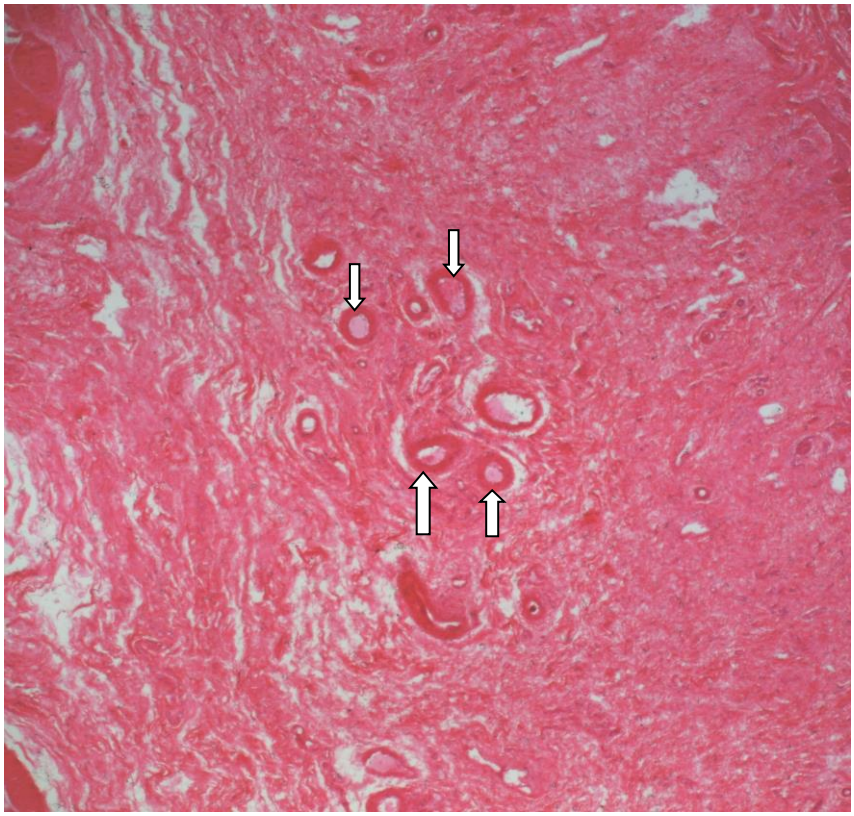

Figure 9: Photomicrograph of the stomach of a slaughtered donkey (20 KNB) showing engorged blood vessels at the submucosa as illustrated by the white block arrows (X40; H&E)

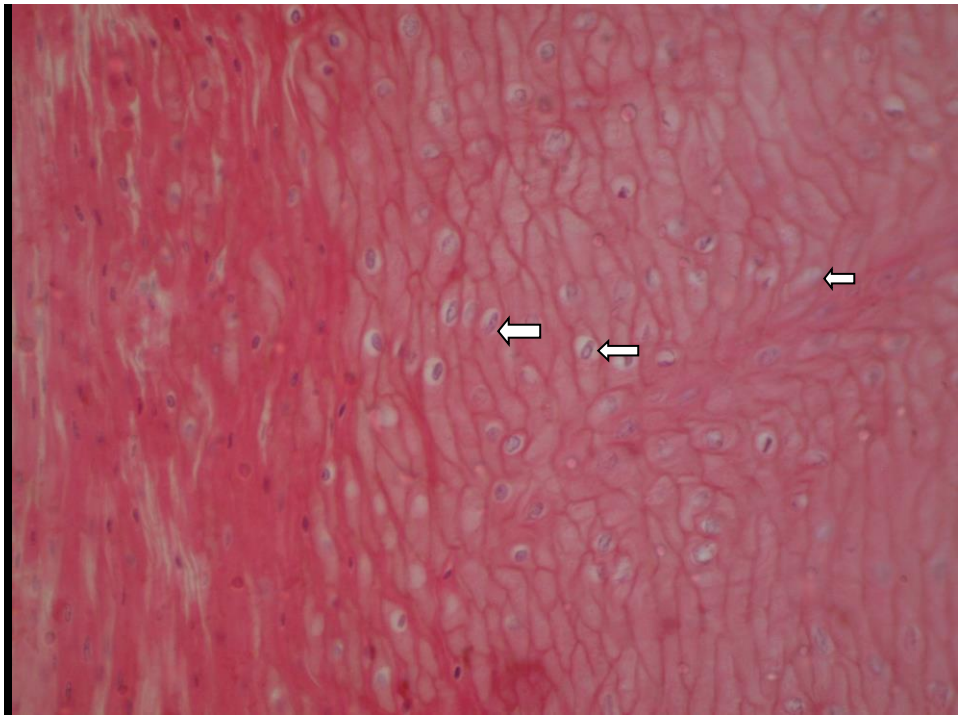

Figure 10: Photomicrograph of the stomach mucosa of a slaughtered donkey (ID: 20KNB) showing vacuolar degeneration (illustrated by the white arrows) in the submucosa of the stomach due to Gasterophilosis (X40; H&E)

4.3.2: Intestines

Anaplocephala magna had an occurrence of 2.5%(7/282) whereas *Anaplocephala perfoliata* **(Figure 11)** had an occurrence rate of 10.3%(29/282). *Cylicocyclus auriculatus* was found in 2.1% (6/282) of the donkeys. Cyathostome species were present in 1.4% (4/282) of the donkeys. *Thelazia equi* was present in one donkey represented at (0.4%) 1/282, *Parascaris equorum* was represented in 20.2% (57/282) of the donkeys, *Strongylus edentatus* at 12.1% (34/282), *Strongylus equinus* at 0.4 % (1/282), *Strongylus vulgaris* at 52.8 % (149/282), *Setaria equina* at 3.5 %(10/282) and *Triodontophorus serratus* at 0.4% (1/282) (**Figure 12**).

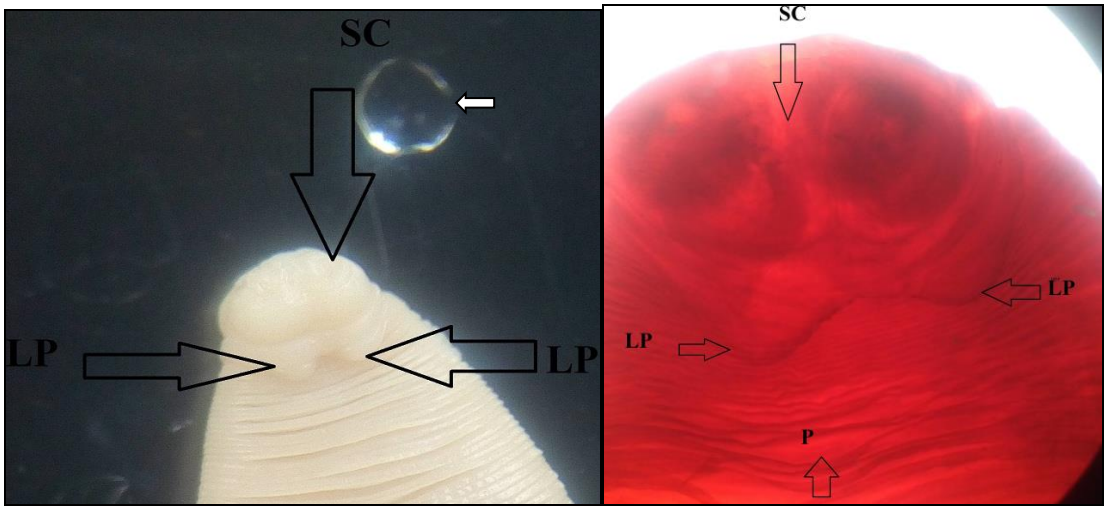

Figure 11 A and B: A photomicrograph of *Anaplocephala perfoliata* recovered from the ileocecal junction of slaughtered donkey (ID: 10 MGT), illustrating the lappets (L) and the scolex (SC) which is distinct and smaller than the body. On the right side is the same cestode after staining with acetoalum carmine stain illustrating the scolex (SC), lappets (LP) and the proglottids (P). The white block arrow shows an artifact (air bubble).

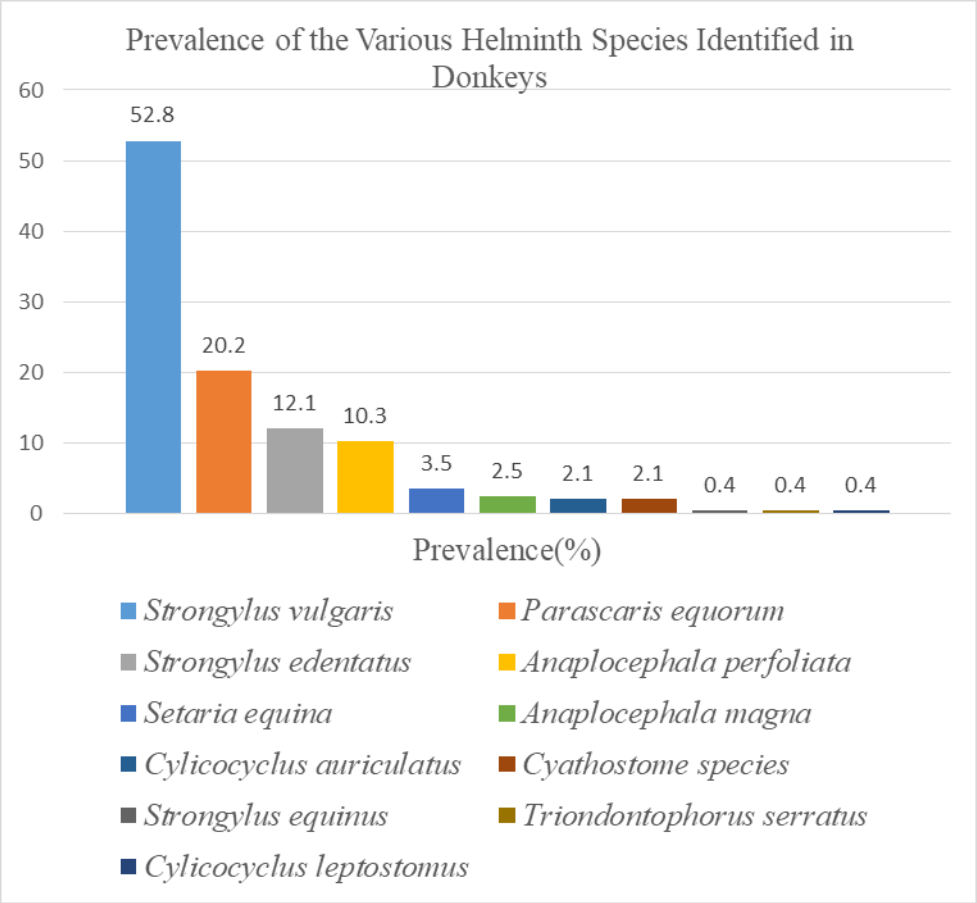

Figure 12: Prevalence of the various helminth species in the intestines of slaughtered donkeys in Kenya

In the ceca of 8 donkeys, focal elevated areas measuring about 2-3mm that were firm in consistency were visible **(Figure 13**). Hyperemia and oedema were also evident on the mucosa. On incision into the cavity a slender reddish worm was observed and identified as *Strongylus edentatus*. Microscopically, there was disruption of the mucosal lining; nematode larval stages were encapsulated within a lumen (**Figure 14**). The outer zone of the capsule was surrounded by fibroblasts and neovascularization was also evident (granulation tissue). The submucosal blood vessels were also dilated (**Figure 15**).There was also a case (ID: 9MGT) with parasite in early developmental stages coupled with eosinophilic infiltration. In some instances there was cellular infiltration in the submucosa characterized by plasma cells, macrophages and eosinophils. The eosinophils seemed to form a vast number of the population as they were also present in the lamina propria. On incision into other elevated

areas on the intestinal mucosa that were firm and whitish in color, a purulent discharge was visible. Microscopically, there was necrotic debri with degenerated neutrophils surrounded by a capsule at the periphery.

Irregular circumscribed hyperemic areas of epithelial loss were seen on the ileocecal junction region after dislodgement of cestodes attached at the region. Microscopically, eosinophils were present in the submucosa with accompanying dilatation of the submucosal blood vessels. Mucosa was disrupted with lymphocytic cellular infiltration, localized areas surrounded by high density fibrous connective tissue and goblet cells were hyperplastic. In another case, there was massive infiltration by macrophages, eosinophils and lymphocytes with accompanying neovascularization in the submucosa. There were instances where the lymph vessels were fully dilated with a cellular infiltration (eosinophils, neutrophils and macrophages). In this case *Strongylus vulgaris* were retrieved from the cecal nodules. There was cecal tissue that had pink eroded areas whose wall was hyperemic and edematous. Numerous *Strongylus vulgaris* species were attached on the mucosa as well **(Figure 16**). On microscopic examination, there was a parasitic larva within the tunica mucosa where the larva was surrounded by fibrous connective tissue with a characteristic eosinophilic infiltration (**Figure 17, 18 and 19).**

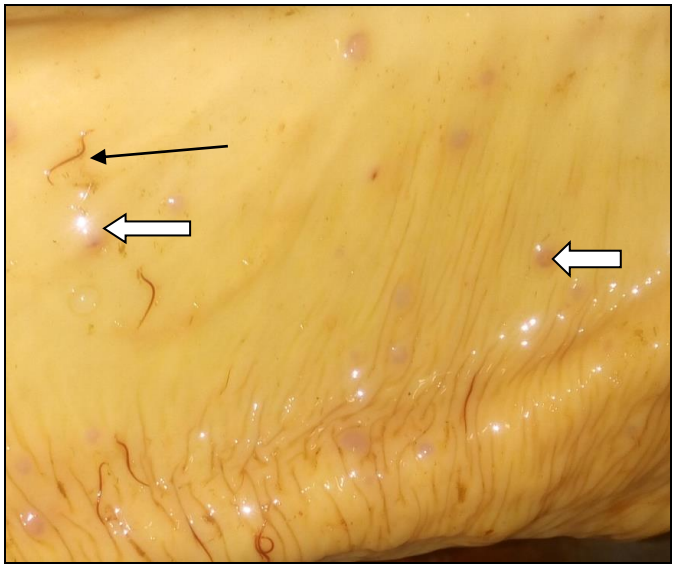

Figure 13: A picture of the cecal wall from slaughtered donkey (ID: 13MGT) showing focal nodular lesions, about 2-5 mm (white block arrow) and a reddish nematode attached onto the mucosa; *Strongylus vulgaris* (thin black arrow).

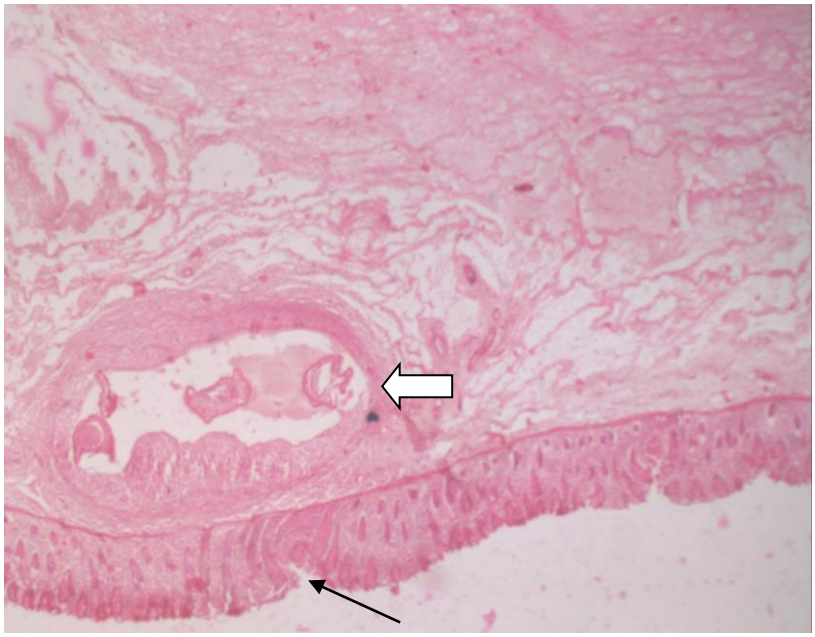

Figure 14: Photomicrograph of the ceca of donkey (13 MGT) showing four regular structures identified as nematode larvae suspected to be larval stages of *Strongylus vulgaris* enclosed in a fibrous capsule(White block arrow) A portion of the cecal mucosa has been disrupted also(Black arrow) (X4).

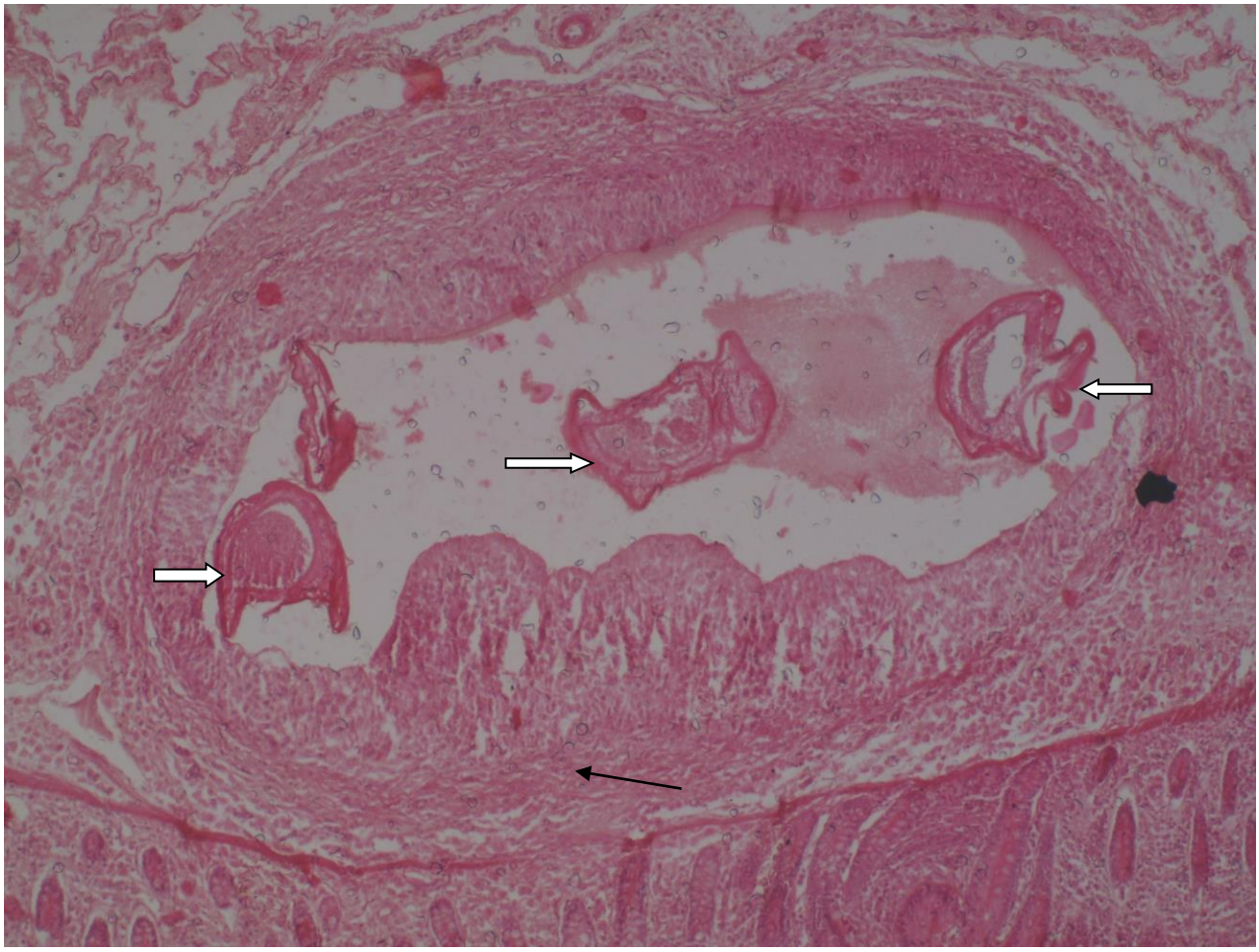

Figure 15: Photomicrograph of slaughtered donkey (ID: 13 MGT) illustrating the fibrous capsule zone (Black arrow) that's encapsulating the nematode larva (white block arrows). The fibrous connective tissue has mixed inflammatory infiltrate (black arrow) (X10; H&E).

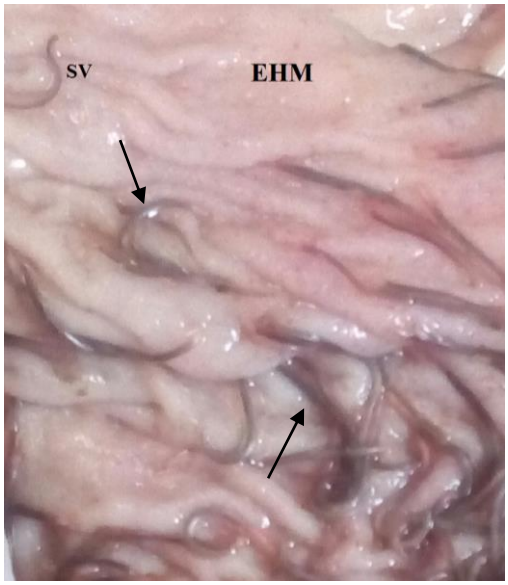

Figure 16: Picture showing attached nematodes on the cecal mucosa (black arrow), *Strongylus vulgaris* (SV). The mucosa is edematous, hyperemic and eroded (EHM) (ID: 3 MGT)

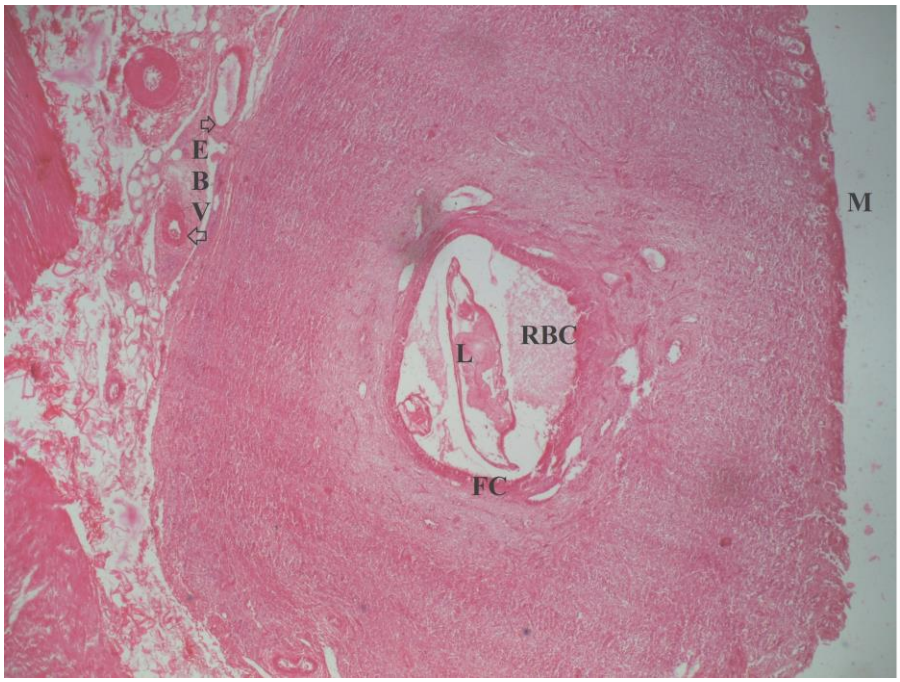

Figure 17: photomicrograph of donkey (ID: 3MGT) illustrating the larvae encapsulated within a lumen (L) with red blood cells adjacent (RBC) and a thin fibrous capsule layer (FC). The mucosa can be seen with a few glands (M), the blood vessels are also engorged with blood (EBV) (X4; H&E)

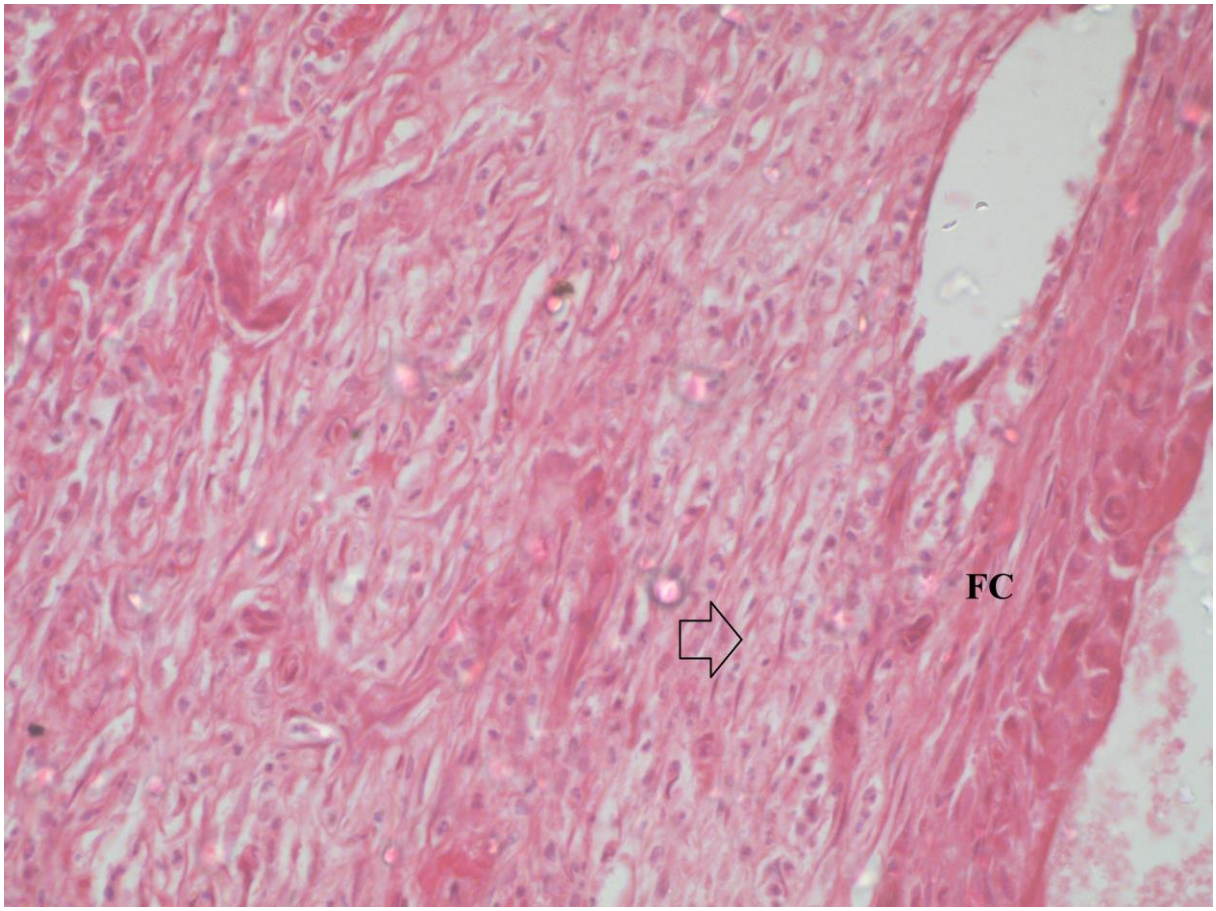

Figure 18: Photomicrograph of donkey (ID: 3MGT) illustrating the mixed inflammatory infiltrate as shown by the arrow and the fibrous capsule (FC) (X40; H&E)

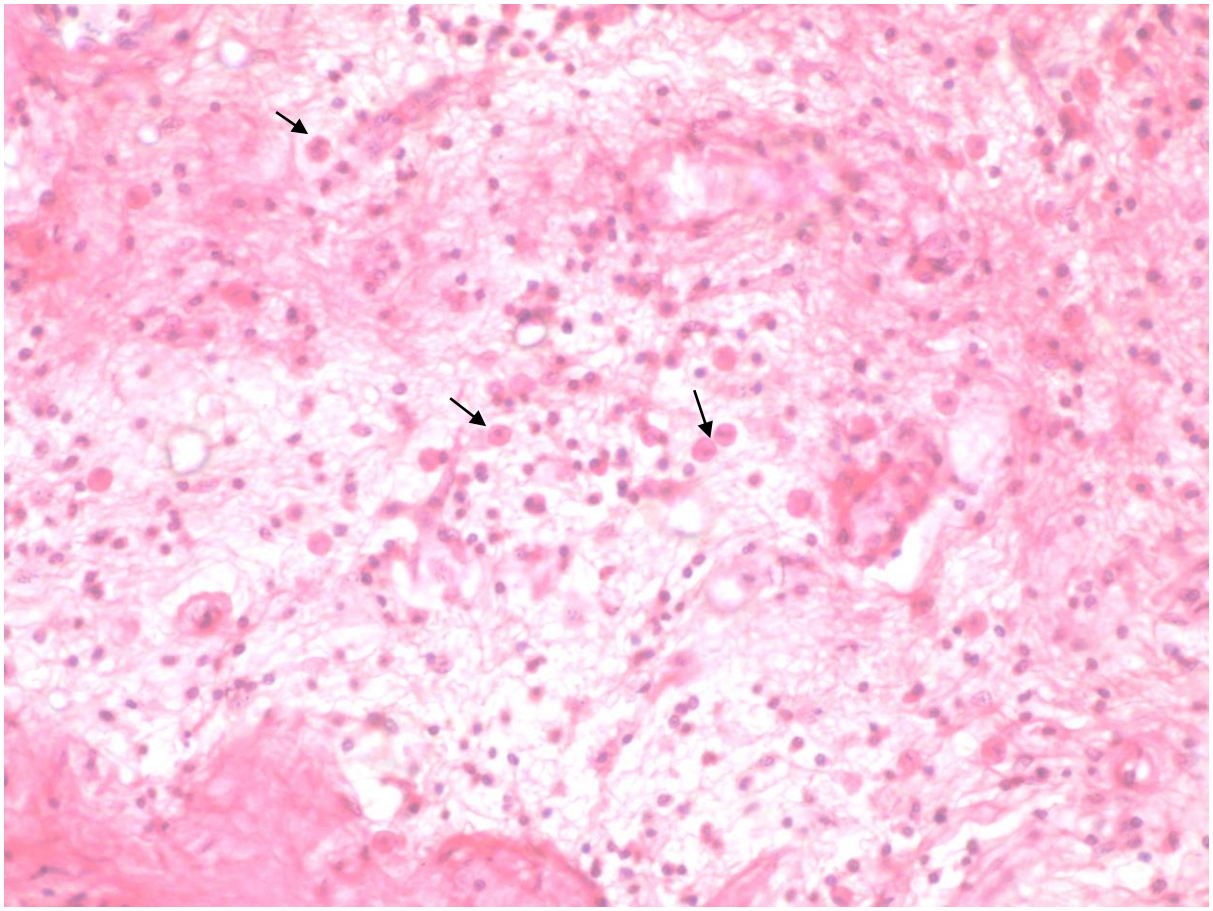

Figure 19: Photomicrograph of donkey ceca showing the submucosa of a donkey (9 MGT) illustrating the mixed inflammatory infiltrate with the eosinophils predominating (black arrow) (X40; H&E)

4.3.3: Liver

In the liver, *Strongylus edentatus* was retrieved. Hepatic enlargement was observed as the margins were rounded in the 15 donkeys sampled and in addition, eight of the liver tissues exhibited cyst-like focal swelling measuring 2-3 cm in diameter that protruded above the capsule **(Figure 20**). On incision, blood filled cavity containing a slender nematode was observed in the eight cases. The nematode was identified as *Strongylus edentatus*. Microscopically, there was a well demarcated zone characterized of hemorrhage, loss of hepatic architecture and brown pigment deposition. In some cases there was a well demarcated zone between the liver and the nodule with red blood cells, necrotic debri filling the area where the helminth resided (**Figure 21).** In some cases, the well demarcated zone had deposition of fibrous connective tissue proliferated bile ducts in the portal triad, cellular infiltration and disrupted sinusoids.

Greyish pinpoint necrotic foci were also observed in one of the donkeys which had a high infestation of *Parascaris equorum*. On microscopic examination, the foci were composed of neutrophils interspersed amongst cell debri at the centre and a fibrous capsule at the margin which was suggestive of hepatic abscessation (**Figure 22 and 23**).

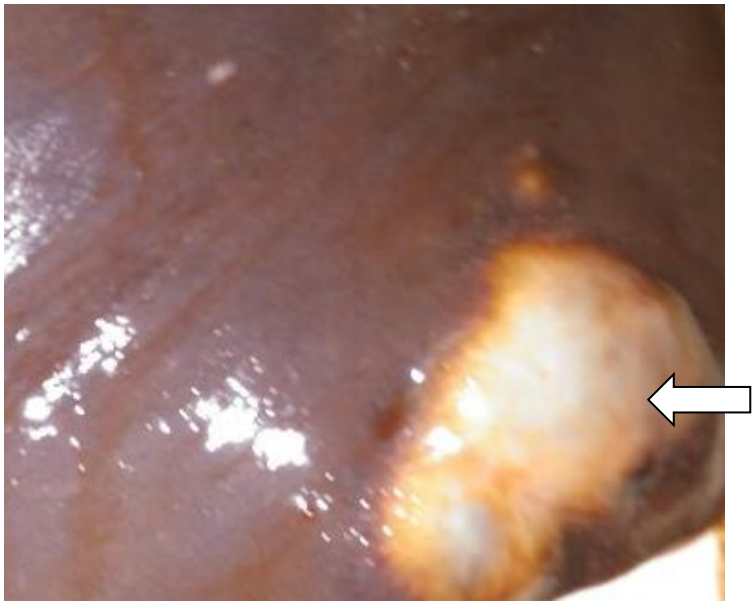

Figure 20: Liver of a slaughtered donkey (ID: 60 MGT) showing the cyst-like swelling (white arrow) protruding above the capsule.

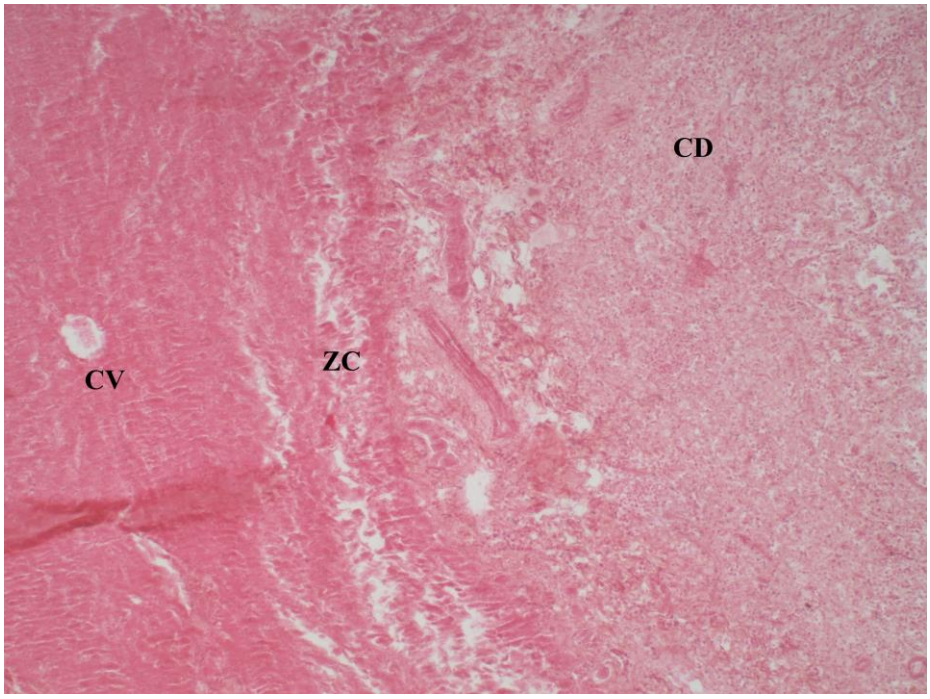

Figure 21: Photomicrograph of the liver of a slaughtered donkey (60 MGT) showing the well demarcated zone (ZC) with the central vein engorged with blood (CV) , the enclosed material(CD) is where the retrieved helminth (*Strongylus edentatus*) was residing and is composed of necrotic material and no hepatic tissue can be appreciated(X10; H&E)

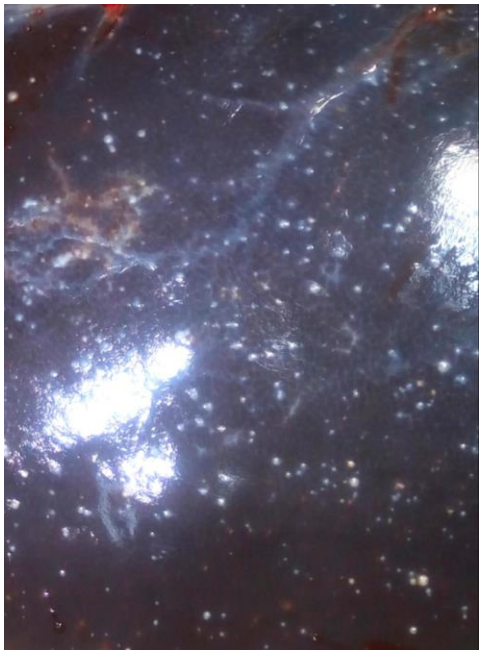

Figure 22: Picture of a liver of a slaughtered donkey (ID: 18 LDW) showing multifocal greyish areas on the liver surface.

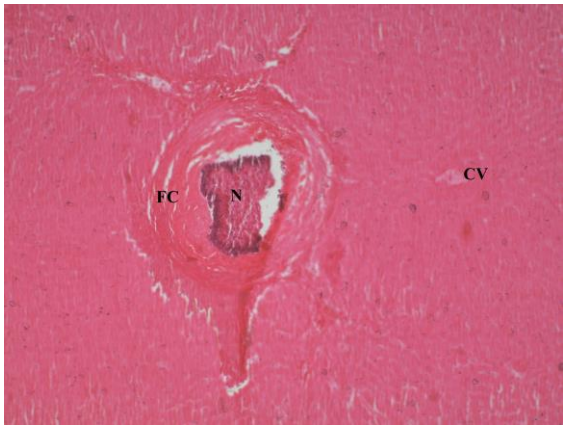

Figure 23: Photomicrograph of a donkey liver (ID: 18 LDW) showing a focal area with necrotic material with interspersed neutrophils (N) and an accompanying fibrous capsule (FC)-Hepatic abscess. The central vein is also illustrated (CV) (X10; H&E).

4.4: Occurrence of Hemoparasites in the slaughtered donkeys

In the 160 buffy coat smears examined, 11.3% (18/160) were positive for the various hemoparasites while 88.8% (142/160) were negative. As for the blood smears, 6.25% (10/160) were positive whereas 93.75% (150/160) were negative (**Table 5**). Mixed infections were represented at 1.25% (2/160) for *Babesia caballi* and *Trypanosome* spps as examined in buffy coat smear.

Table 5: Table showing the number of positive cases for thin blood smears and the buffy coat smears from slaughtered donkeys from the three slaughter houses

Hemoparasite identified	**No. of donkeys positive on thin blood smears examination**	**No. of donkeys positive on buffy coat smears examination**
Anaplasma spp	0	4
Trypanosoma spp	1	9
Babesia caballi	8	4
Microfilariae	0	1
Theileria equi	1	0
Total number of positive donkeys	10/160	18/160
Overall Proportion (%)	6.25%	11.3%

4.4.1: Buffy Coat Smears

Anaplasma spp had an occurrence of 2.5%(4/160), trypanosomes at 5.6%(9/160), *Babesia caballi* at 2.5%(4/160) and microfilaria at 0.60%(1/160) (**Figure 24**). Photomicrographs of the various hemoparasites have been illustrated (**Figure 25,26 and 27**).

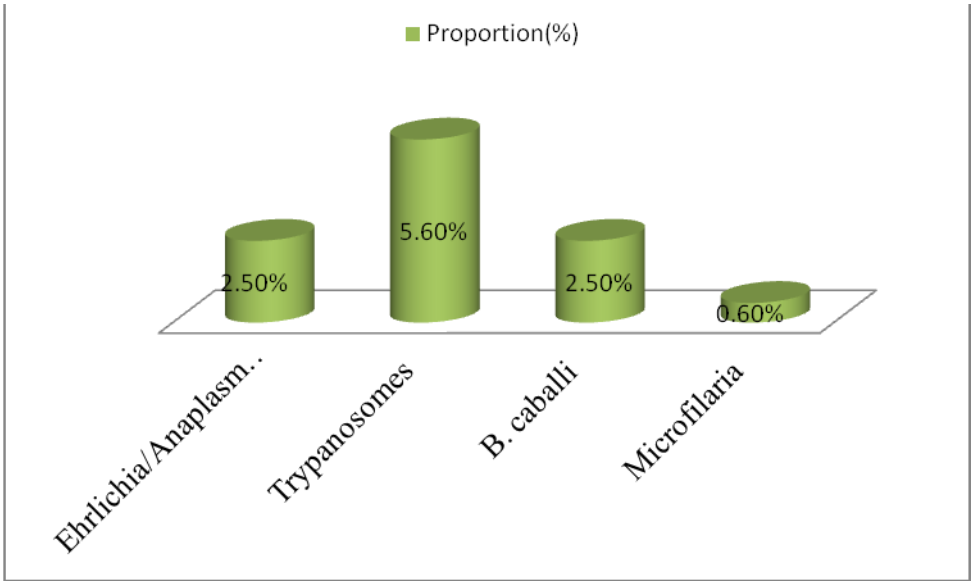

Figure 24:Occurrence of Hemoparasites as Examined on the Buffy coat smears of slaughtered donkeys

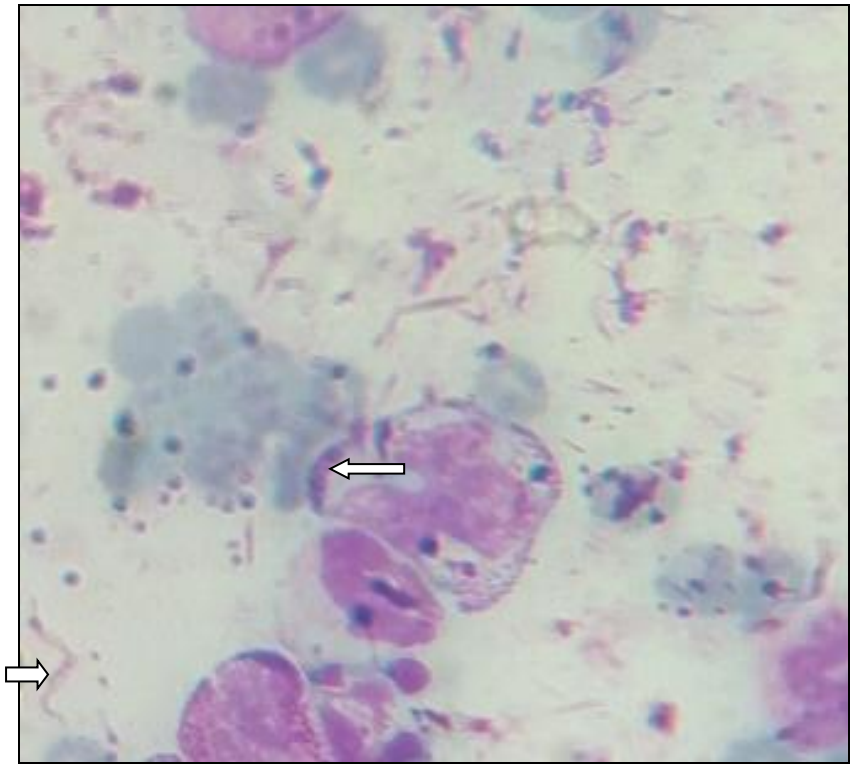

Figure 25: Photomicrograph of a buffy coat smear of animal (ID: 2LDW) showing *trypanosome* spp (white block arrows) in a donkey, (X100;Giemsa Stain)

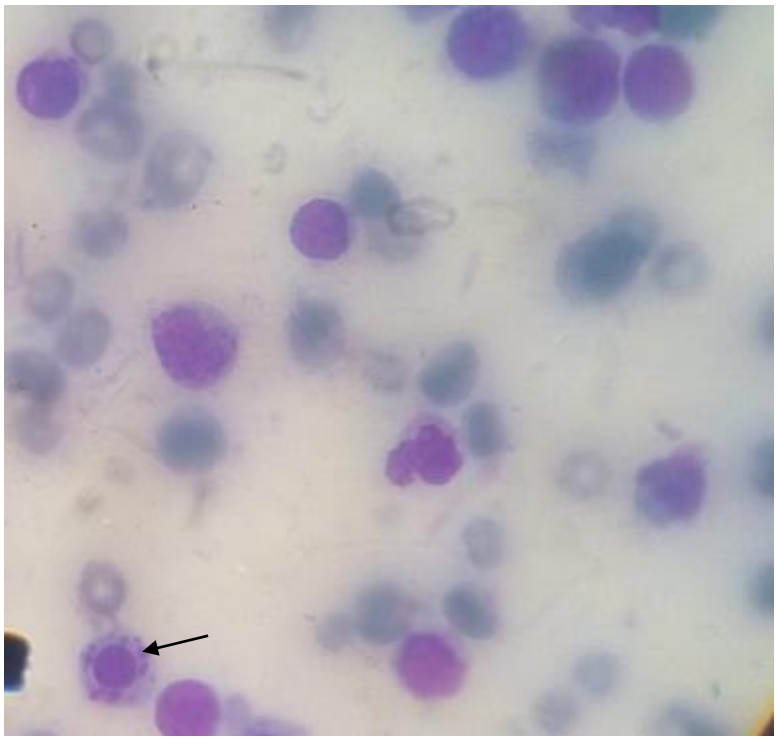

Figure 26: Photomicrograph of a buffy coat smear from donkey (ID: 4LDW) showing a lymphocyte with purple staining inclusions at the margin (*Anaplasma* spp-Black arrow). (X100;Giemsa Stain)

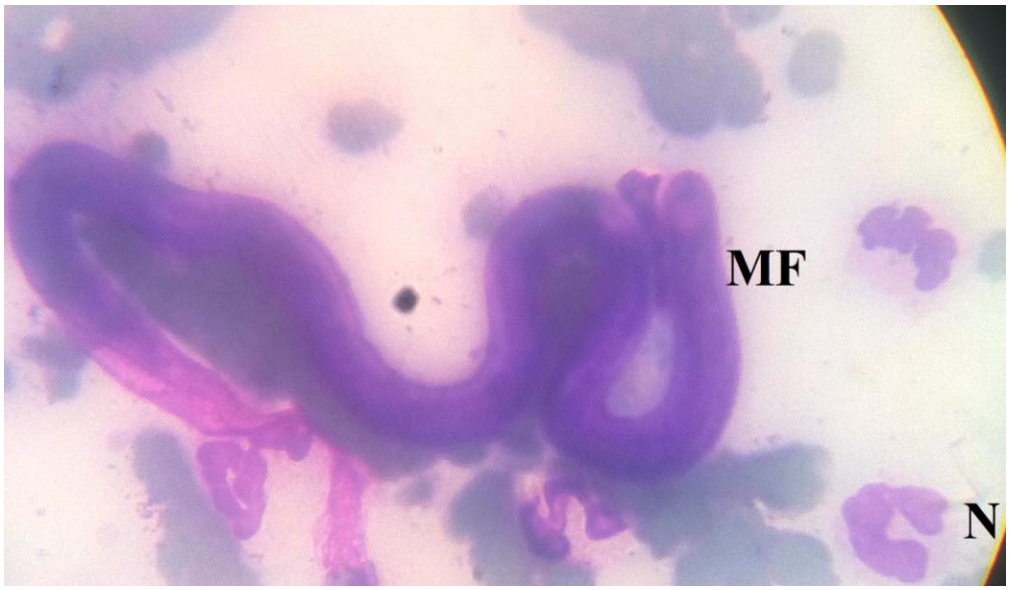

Figure 27: Photomicrograph of a buffy coat smear of a slaughtered donkey(ID: 80KNB) showing *Setaria equina* microfilariae(MF), a band neutrophil(N) (X100;Giemsa Stain)

4.4.2: Thin Blood Smears

In the 160 animals that were screened, *Babesia caballi* had an occurrence of 5% (8/160), *Theileria equi* with an occurrence rate of 0.6% (1/160) *Trypanosoma* spps at an occurrence rate of 0.6% (1/160) as illustrated in Table 6. Some of the hemoparasites identified have been shown also **(Figure 28, 29, 30 and 31**).

Table 6:Occurrence of Hemoparasites as Examined on the Thin Blood Smears from slaughtered donkeys

Hemoparasite	Frequency	Proportion (%)
Babesia caballi	8/160	5%
Theileria equi	1/160	0.60%
Trypanosoma spps	1/160	0.60%

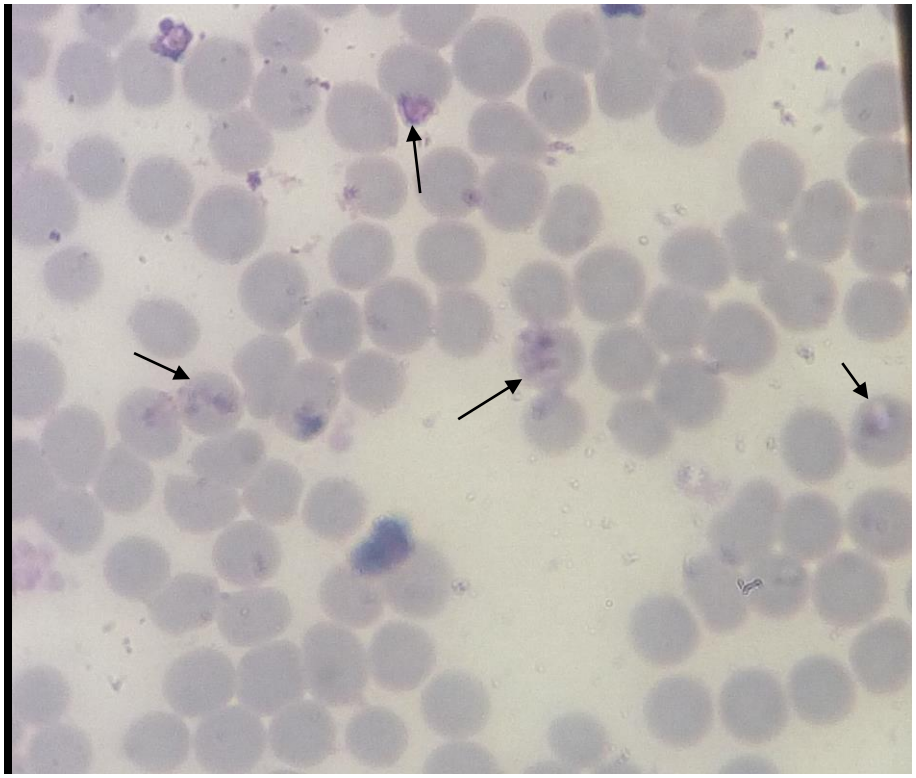

Figure 28: Photomicrograph of a thin blood smear from donkey (ID: 4LDW) showing red blood cells with two pyriform shaped inclusions black arrow(*Babesia caballi*). Note the red blood cells that are polychromatophilic with varying shapes and sizes.(X 100;Giemsa Stain)

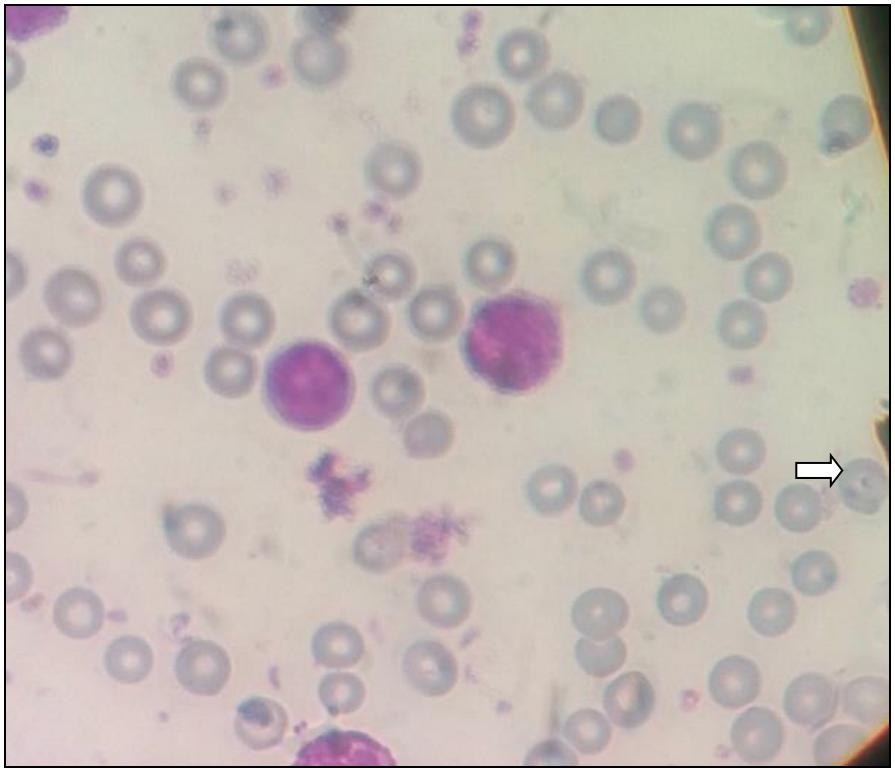

Figure 29: Photomicrograph of a buffy coat smear from donkey (ID: 78 KNB) showing a red blood cell with *Babesia caballi* (X100 Giemsa stain)

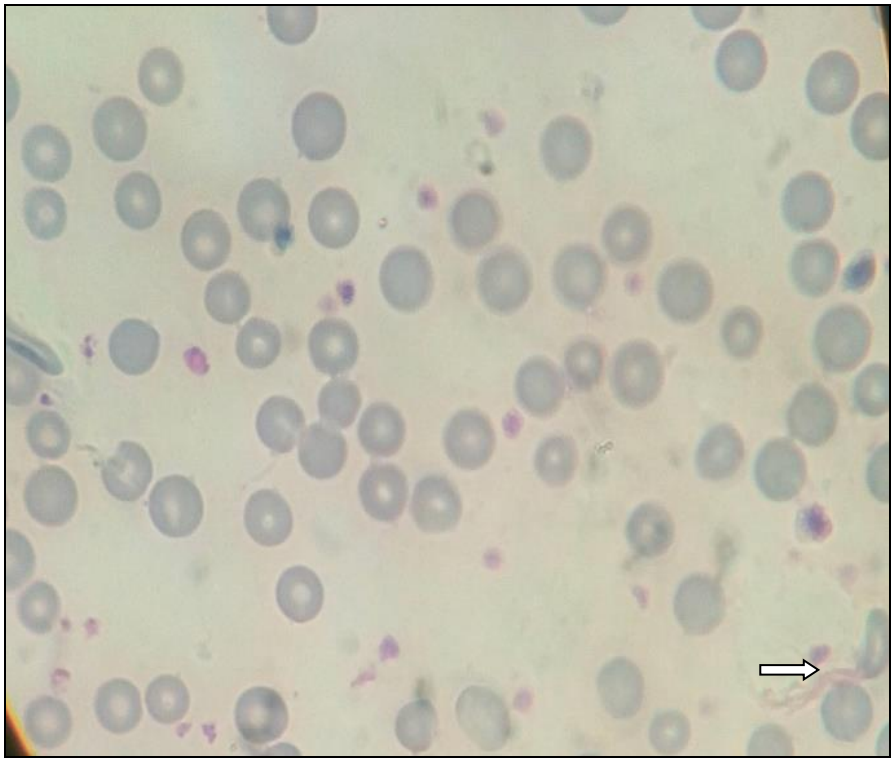

Figure 30: Photomicrograph of a buffy coat smear from a donkey (ID: 78KNB) ***trypanosome*** **spp can be seen (White arrow head) (X100; Giemsa Stain).**

The various cellular morphological alterations associated with the hemoparasites included; poikilocytosis, anisocytosis, polychromasia, hypersegmented neutrophil and reactive lymphocytes (**Figure 31**) (**Table 7**).

Table 7: Cellular Morphological Alterations due to Hemoparasites on slaughtered donkey blood smears in Kenya

Hemoparasite(s)	Associated Morphological Alterations
Trypanosoma spps	Poikilocytosis(echinocytes) and anisocytosis
Babesia caballi	Poikilocytosis(echinocytes),anisocytosis,polychromasia and hypersegmented neutrophil
Babesia caballi	Poikilocytosis(echinocytes), anisocytosis and hypersegmented neutrophil
Babesia caballi	Poikilocytosis(echinocytes) anisocytosis, polychromasia and reactive lymphocyte
Theileria equi	Poikilocytosis(echinocytes) anisocytosis and polychromasia

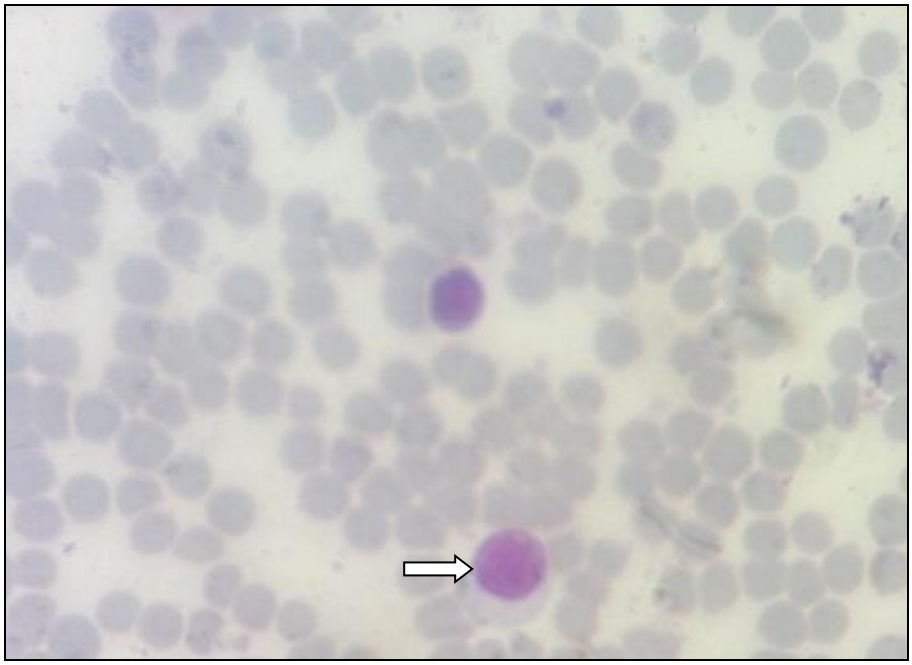

Figure 31: Photomicrograph if a thin blood smear from donkey(ID: 69 MGT) illustrating a lymphocyte with vacuolated cytoplasm(white arrow head) and the variation in shape and size of the red blood cells can also be appreciated(X100; Giemsa Stain).

5.0: CHAPTER FIVE: DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1: DISCUSSION

5.1.1: Proportion and Intensity of Endoparasites Infestation in Donkeys

According to this study, parasitism was found to be a prevalent condition (85.5%) amongst donkeys in Kenya. This is supported by similar studies done by Karanja *et al.* (1994) and Lewa *et al*. (2000) that showed gastrointestinal parasites are prevalent in donkeys in Kenya. This differed a little to a study done in Ethiopia where the prevalence was 96.9% (Ibrahim *et al*., 2011). This difference could be attributed to differences in geographical zones and management systems as well. Majority of the donkeys in this study (89%) had a poor body condition. This could be due to helminthosis in conjunction with a low plane of nutrition. This is in agreement with (Saul *et al*., 1997) who reported helminth infestation as the most common cause of death besides retarding growth or decreasing work output in addition to distress and pain (Svendsen, 1997). Lewa *et al.* (1998) also noted that the unthrifty state in donkeys was caused by heavy helminth infections, majorly the adult worms. An overall of 10 adult helminth species obtained included; *Anaplocephala magna* 2.5%(7/282), *Anaplocephala perfoliata* 10.3%(29/282), *Cylicocyclus auriculatus* 2.1%(6/282), *Cylicocyclus leptostomus* 0.4%(1/282), *Cyathostome* species 2.1%(6/282), *Parascaris equorum* 20.2% (57/282), *Strongylus edentatus* 12.1%(34/282), *Strongylus equinus* 0.4 % (1/282), *Strongylus vulgaris* 52.8 %(149/282), *Setaria equina* 3.5% (10/282) and *Triodontophorus serratus* 0.4 %(1/282).This finding differs with Ahmed *et al.* (2011) who found the overall prevalence to be 98.5% with 22 adult helminth species and this could be attributed to the different geographical regions and management systems. The most common species in donkeys in our study was the *Strongylus vulgaris* (52.8%). Lewa *et al.* (2001) found *Gastrophilus intestinalis, Strongylus vulgaris* and *Strongylus edentatus* in the six donkeys examined at necropsy. *Gasterophilus nasalis, Cyathostomum tetracanthum,*

Habronema muscae, Strongylus spp*, Setaria equina* and *Oxyuris equi* were the common parasites found in eight donkeys in Kenya (Karanja *et al*., 1994).This study has revealed more other species occurring in donkeys in Kenya which include *Triodontophorus serratus, Cylicocyclus auriculatus, Cylicocyclus leptostomus Parascaris equorum*, *Anaplocephala magna* and *Anaplocephala perfoliata*. *Habronema species* eggs were also observed in two donkeys in our study. The additional species could be due to the larger sample size used in this study and also the donkeys were mostly from the arid and semiarid areas of Kenya contrary to the previous study in which the donkeys were from Kiambu County only. Getachew *et al.* (2010) observed 8% prevalence of *Anaplocephala perfoliata* in working donkeys in Ethiopia and this is almost similar to the prevalence found in this study (10%).

Fecal examination in this study revealed that 44.7 % (126/282) of the donkeys were positive for strongyle eggs, *Parascaris equorum* occurred in 5.3% (15/282) of the donkeys followed by *Oxyuris equi* at 1.1 %(3/282), *Triondontophorus tenuicolis* and *Habronema* spp occurred each at 0.7 %(2/282) infection rate and cestode eggs which were present in only one donkey at 0.4 %(1/282). According to a report by Ahmed *et al* (2011) in Ethiopia, fecal examination showed 99% strongyle, 80% *Fasciola*, 51% *Parascaris,* 30% *Gastrodiscus,* 11% *Strongyloides westeri*, 8% *Anaplocephala perfoliata* cestodes and 2% *Oxyuris equi* infection prevalence. The infection level in this study was lower and this could be attributed to the seasons in Kenya as the animals were sampled during the fairly dry season. Additionally, donkeys originated mostly from the dry areas and these include Isiolo, Loitoktok, Laikipia, Tana River, Suswa-Narok, Kajiado, Narok, Kitui and Karamoji (Uganda). Some animals also originated from Kericho. According to Lewa *et al.* (1998) egg counts seemed to be relatively low during the dry season. The egg count for the strongylid eggs ranged from 0-1900, which is low as compared to that of Vercruysse *et al.* (1986) who established an egg count of 100-9,200 for strongylid eggs. Karanja *et al.* (1994), in Kenya, found the infection to be low to

moderate for strongyle infestation, this is in agreement with the results of this study as most donkeys had an egg count of 0-499 EPG. The number of adult helminths observed was however high and this shows that it does not correlate to the fecal egg count. As observed in this study, a fecal egg count of zero did not mean the donkey was parasite free. Additionally, the animal could be harboring a majority of the immature stages of the helminths in high numbers hence the resultant low/no egg count. This is an agreement with an observation made by Matthews and Burden (2013), who stated that donkeys can harbor substantial levels of parasitic infection but the parasites aren't detectable via routine faecal egg count (FEC) analysis.

5.1.2: Risk Factors to Occurrence of Helminths and Strongyle Egg Count

There was no significant differences in strongyle egg count between the adults and the juveniles ($P>0.05$). This is in contrary to the study in Lesotho which indicated an inverse association between age and intensity of strongyle infection (Upjohn *et al*., 2010, Yoseph *et al*., 2005). Additionally no significant differences were observed in strongyle eggs shed between the males and the females. This could suggest that the management of the animals at the farm level is the same. This is in contrary to the study in Lesotho where a significant strongyle infection for females was observed by Upjohn *et al.* (2010). Variations in the husbandry practices between the age and sex were however not determined and this requires further investigation. A significant difference was seen between the strongyle eggs shed and body condition scores ($P< 0.05$). Yoseph *et al*. (2005) reported that animals with a poor body condition had a high fecal egg count. Ibrahim *et al.* (2011) also found out that donkeys with a poor body condition score had a high prevalence of helminth parasites as compared to well-conditioned donkeys. This is agreement with this study that showed a high prevalence of strongyle egg counts in poor body conditioned animals. A recent study carried out in Morocco by Crane *et al.* (2011) showed that considerable increase in body condition score in

the equids that received anthelmintic treatment contrary to those in which a placebo was administered. With regard to the prevalence of the helminths there was no association found between the body condition score and the presence of the helminths in our study. This is in agreement with a recent study done by Tesfu *et al.* (2014) who reported no association of nematode infection with regard to body condition score of the donkeys. In this study, both body condition score groups were equally affected and could mean that body condition score of 2 or less could indicate a need for therapeutic intervention with antihelminthic drugs.

A nonparametric correlation test to determine the presence of helminth parasite in the age and sex in this study revealed that there was no association between these two factors and the presence of helminths. This is in agreement with Ibrahim *et al.* (2011) who found that all age groups were equally affected. For sex however, the females were found to be mostly affected as compared to males (Attia *et al*., 2018).This differs with this study and this could be because both male and female donkeys are raised in the same environmental conditions.

5.1.3: Occurrence of Hemoparasites

A higher occurrence of *Trypanosome* spps were observed under the buffy coat smears(5.6%) as compared to the thin blood smears((0.6%).This denotes that buffy coat smears were more sensitive in detecting the *Trypanosome* spps. A proportion of 10.7 % of the donkeys in South Ethiopia were positive for trypanosomes (Mekuria *et al*., 2010). This is a slightly higher prevalence than the findings in this study as the method used in the latter was dark ground and phase contrast buffy coat method (Mekuria *et al*., 2010). In this study, *Theileria equi* had an occurrence of 0.6% whereas *Babesia* spps had an occurrence of 5%. This is in contrary to a recent study done in Northern part of Kenya by Hawkins *et al* (2015), where 72% of the

donkeys were positive for *Theileria equi* whereas no animal was positive for *Babesia caballi.* This variation could be explained by the technique used; PCR which is highly sensitive.

Microfilaraemia in equine in Baghdad was reported to have an occurrence rate of 11.11% via use of Knott technique and species were identified as those of *Setaria equina* (Afkar and Amall, 2014). In Iraq the percentage of infection amongst horses was 30.76% via use of Knott technique (Suleiman *et al*., 2012). The occurrence rate in this study was at 0.6% which is low and could be due to the standard technique used to screen for the hemoparasite and also differences in occurrences could be due to the different geographical areas.

5.1.4: Characterization of Lesions Associated with Helminths in donkeys

5.1.4.1: Stomach

Perforation or rupture of the gastrointestinal tract leading to peritonitis has been documented as consequence of *Gasterophilus* infestation (Van der Kolk *et al.,* 1986). Ulcers frequently occur in the non-glandular mucosa of the stomach which doesn't have adequate protection against the detrimental effect of stomach acids (Andrews *et al*., 2005). In our study widespread areas of erosion and ulcers were seen after dislodging the various species of *Gasterophilus species* in the non-glandular region. The bots significantly affected the non-glandular region. This was also observed by Al-Mokaddem *et al*. (2015) in Egypt where they found tiny superficial erosions upon removal of the larvae. Microscopic vacuolar degeneration of the squamous cells, thickened keratinized layer of the stomach lining were observed in this study. According to Al-Mokaddem *et al.* (2015) the microscopic examination showed the attachment site appearing like deep, pitted ulcer with exposed lamina propria, granulation tissue formation and neutrophilic infiltration. Additionally, they found; hyperkeratosis, acanthosis, vacuolar degeneration of stratified squamous cells, gastritis,

erosions, ulcerations, scarring, hyperactivity of mucus glands, periglandular fibroplasia and parasitic granulomes with infestation by *Gasterophilus* larvae.

This current study revealed a similar pathological pattern whereby the mucosal epithelium was thickened and there was loss of tissue architecture. Additionally there was disruption of the keratinized layer of the non-glandular stomach. The keratin covering the surface epithelium however had a yellow tinge and this could be due to bile as the donkeys were starved in the lairages prior slaughter. Empty white spaces were visible in the lamina propria (vacuolar degeneration).

5.1.4.2: Intestines

The mucosa of the intestines had nodules on gross examination and was covered with mucoid exudate. On incision, a worm was retrieved in most cases. Microscopically, there was dilation of the submucosal blood vessels with four regular structures suspected to be larva of *Strongyle* spp encapsulated by granulation tissue. This was also accompanied by a high cellular infiltration characterized by eosinophils, neutrophils and macrophages within the sub-mucosa. This is similar to observations by Lewa *et al.* (2000) who found out that both the large and small strongyles cause enteritis with the mucosal surface having parasitic nodules. Microscopically, the mucosa and the sub-mucosa had cellular infiltrations. Penetration of the third stage larvae of *Strongylus vulgaris* causes small hemorrhages through the intestinal wall. The small nodules could be due to *Strongylus edentatus* owing to its life cycle in the liver and also the intestines. A similar study done by (Lewa, 1998), showed that the 3rd stage larvae of *Strongylus edentatus* was partially the cause of the nodules observed in the small intestines. The larvae go through the wall of the intestines and reach the liver via portal veins where they grow to 4th stage which migrate within the liver and also contribute to necrosis and inflammation. The nodules in this current study were majorly affecting the ceca. Cyathostomes are obtained by horses within pasture which upon ingestion, primarily go

through a period of arrested development in the mucosa, submucosa, or both, of the large intestine and cecum as well. Their emergence has been associated with ill effects including diarrhea, emaciation and instigates an inflammatory protein-losing enteropathy and changes in intestinal motility (Love *et al*., 1999). As the cyathostomes are emerging they cause rupture of the muscularis mucosae, local eosinophilia, edema and infiltration by macrophages and neutrophils as well (Soulsby, 1982). One of the life threatening parasites is the small strongyles that cause larval cyathostominosis and usually encyst into the large intestine and their larvae can initiate severe damage in the lining of the intestine (Oryan *et al*., 2015) .The findings in our study could imply that there was a possibility of mixed infection by both the small and large strongyles as both categories of helminths were identified. Oryan *et al.* in (2015) established that the lesions in the caecum of a six year old working donkey were associated with non-suppurative enteritis with characteristic infiltration of eosinophils, plasma cells, lymphocytes and macrophages in the intestinal mucosa, submucosa and lamina propria. The parasites identified on parasitological examination revealed two species of cyathostomes that included *Cylicocyclus elongatus* and *Cyathostomum pathratum.*

Stress, interconnected with work and inadequate nutrition frequently results in a loss of condition and cyathostomid nematodes may then bring about the clinical disease (Krecek and Guthrie, 1999).

Lesions associated with *Anaplocephala perfoliata* observed in this study included; grossly mucosal erosions were observed, lymphocytic cellular infiltration, localized area that was surrounded by fibrous connective tissue and hyperplastic goblet cells. Sangioni *et al.* (2000) reported macroscopic and microscopic lesions linked to *Anaplocephala perfoliata* which included mucosal erosion, ulcerations, edema accompanied by eosinophilic and mononuclear cell infiltration in the mucosa and the basal membrane of the caecum and colon. It also causes

hypertrophy of the intestinal wall at the site of attachment and can as well cause perforation leading to colic (Soulsby, 1982).

5.1.4.3: Liver

In this study, tiny focal necrotic lesions and hepatomegally were seen in liver samples grossly. On microscopic examination chronic hepatitis was observed. Hepatic abscessation was also observed in donkeys that had an infestation with *Parascaris equorum*. The same animal was positive for *Strongylus vulgaris*. Similar findings were found in livers of donkeys examined by Lewa *et al.* (2000) who observed traumatic hepatitis, bile duct thickening and abscess formation which were linked to *Strongylus species* larvae. Hepatic lesions have also been associated with migrating larval stages of *Parascaris equorum* (Maxie *et al.,* 2016). Hepatic hemorrhagic nodules were seen in livers of eight donkeys. In our study, hemorrhagic nodules due to *Strongylus edentatus* was common. *Strongylus edentatus* has been associated with hepatic hemorrhagic nodules in the liver associated with the 4^{th} stage larvae 3-5 months post infection (Soulsby, 1982). Migration of *strongylus equinus* through the liver also causes formation of nodules and formation of fibrous tissue. An acute reaction can also result in the liver due *Strongylus edentatus* infection (Johnstone, 1998).

5.2: CONCLUSION AND RECOMMENDATIONS

5.2.1 CONCLUSION

1. Donkeys in Kenya are affected by a variety of gastrointestinal helminth parasites and hemoparasite species.

2. The prevalence of gastrointestinal parasites is high in donkeys (85.5%) in Kenya and interferes with their work performance as a majority of them (89%) had a poor body condition.

3. These parasites cause significant pathological lesions in the gastrointestinal tract and liver.

4. These lesions interfere with the normal functioning of the body resulting in lowered meat and skin quality which are a prime cut in the donkey value chain enterprise.

5. Risk factors such as poor body condition scores predispose the donkeys to gastrointestinal parasites

5.2.2 RECOMMENDATIONS

1. Control measures for gastrointestinal parasites and hemoparasites in donkeys is recommended so as to ensure the wellbeing of these animals.

2. Further studies on the effects of the hemoparasites on the performance of donkeys in Kenya should be carried out in areas where the donkeys are raised. Such study should involve larger sample sizes and sensitive diagnostic techniques should be applied.

3. Further study on zoonotic parasites in donkeys is recommended.

4. Further hematological studies in donkeys should be done.

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7.0: APPENDICES

Appendix 1: Field Data Collection Sheet

Name of Abattoir:	**Source of animals:**		
Average no. of animals slaughtered/day:			
Animal Id	**Approximate Age**	**Sex**	**Comments(weakness, approximate body condition score, pregnancy status, gross lesions observed)**

Appendix 2: Body Condition Scoring System for Donkeys

CONDITION	NECK AND SHOULDERS	WITHERS	RIBS AND BELLY	BACK AND LOINS	HINDQUARTERS
1. POOR	Neck thin, all bones easily felt. Neck meets shoulder abruptly, Shoulder bones felt easily, angu-Lar	Dorsal spine of withers Prominent and easily felt	Ribs can be seen from a distance and felt with ease. Belly tucked up.	Backbone prominent, can feel dorsal and transverse processes easily	Hip bones visible and felt easily (hock and pin bones). Little muscle cover. May be cavity under tail.
2. MODERATE	Some muscle Development Overlying bones. Slight step where neck meets Shoulders	Some cover over dorsal withers, spinous processes felt but not prominent	Ribs not visible but can be felt with ease	Dorsal and transverse processes felt with light pressure. Poor muscle development either side midline.	Poor muscle cover on hindquarters, hip bones felt with ease.
3. IDEAL	Good muscle development, bones felt under light cover of muscle/fat. Neck flows smoothly into shoulder, which is Rounded	Good cover of muscle/fat over dorsal spinous processes withers flow smoothly into back	Ribs just covered by light layer of fat/ muscle, ribs can be felt with light pressure. Belly firm with good muscle tone and flattish outline.	Cannot feel individual spinous or transverse processes. Muscle development either side of midline is good	Good muscle cover in hindquarters, hip Bones rounded in appearance, can be felt with light Pressure
4. FAT	Neck thick, crest hard, shoulder covered in even fat layer	Withers broad, bones felt with firm Pressure	Ribs dorsally only felt with firm pressure, ventral ribs may be felt more easily. Belly overdeveloped.	Can only feel dorsal and transverse processes with firm pressure. Slight crease along midline	Hindquarters rounded, bones felt only with firm pressure. Fat Deposits evenly placed
5. OBESE	Neck thick, crest bulging with fat and may fall to one side.	Withers broad, unable to feel bones	Large, often uneven fat deposits covering dorsal and possibly ven-	Back broad, unable to feel spinous or transverse processes.	Cannot feel hip bones, fat may overhang either side of tail

	Shoulder rounded and bulging with fat.		tral aspect of ribs. Ribs not palpable. Belly pendulous in depth and width.	Deep crease along midline bulging fat either side.	head, fat often uneven and bulging

Appendix 3: Analyzed Results

Independent Samples t-Test(Strongyle egg count between adults and juveniles)

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	T	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
Strongyle eggs	Equal variances assumed	1.644	.201	1.075	280	.283	63.907	59.449	-53.117	180.930
	Equal variances not assumed			1.396	19.928	.178	63.907	45.780	-31.611	159.425

No significant difference in strongyle egg count between adults and juveniles, p>0.05

Independent Samples t-Test (Strongyle egg count between males and females)

	Levene's Test for Equality of Variances	t-test for Equality of Means

		F	Sig.	T	Df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
Stronyle eggs	Equal variances assumed	.256	.614	-.630	280	.529	-18.202	28.872	-75.035	38.631
	Equal variances not assumed			-.634	246.822	.527	-18.202	28.731	-74.791	38.387

No significant difference in strongyle eggs shed between males and females p>0.05

Group Statistics: Body condition score versus strongyle egg count

	Body condition score	**N**	**Mean**	**Std. Deviation**	**Std. Error Mean**
Strongyle egg count	1	250	154.0000	247.07932	15.62667
	2	32	.0000	.00000	.00000

Independent Samples Test for strongyle egg counts between the Poor and moderate body condition scores

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	T	Df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper

Strongyle egg count	Equal variances assumed	28.812	.000	3.520	280	.001	154.00000	43.74583	67.88754	240.11246
	Equal variances not assumed			9.855	249.000	.000	154.00000	15.62667	123.22270	184.77730

Significant difference in strongyle eggs shed between the two body condition scores

Chi-Square Tests(Association between body condition scores and prevalence of helminths)

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	.121[a]	1	.728		
Continuity Correction[b]	.007	1	.935		
Likelihood Ratio	.125	1	.723		
Fisher's Exact Test				1.000	.488
Linear-by-Linear Association	.120	1	.729		
N of Valid Cases	282				