

**GASTROINTESTINAL PARASITES INFESTING GREVY'S ZEBRA (*EQUUS
GREVYI*) IN THE SAMBURU LANDSCAPE IN SAMBURU COUNTY**

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Sciences of Kenyatta University**

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DECLARATIONS

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

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DEDICATION

This work is dedicated to my parents who created a suitable environment during the whole period of work. I also dedicate it to my kind family members including my brothers and sisters for their support and encouragement.

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ACRONYMS AND ABBREVIATIONS

AWF	African Wildlife Foundation
EPG	Egg per gram of feces count
FEC	Faecal egg count
GIT	Gastrointestinal tract
GPR	General Purpose Reagent
GPS	Geographical Positioning System
IUCN	International Union for Conservation of Nature
KWS	Kenya Wildlife Services
RPM	Revolution per Minute
STH	Soil Transmitted Helminthes
WHO	World Health Organization
RSA	Republic of South Africa

ABSTRACT

The Grevy zebra (*Equus grevy's*), also known as the imperial zebra is the largest wild equid of the three species of zebras. The other two are the plain zebras (*Equus quagga*) and the mountain zebras (*Equus zebra harmannae*). It belongs to the class mammalia, order perissodactyla and its family is Equidae. Among the three, it is also rated as the most threatened species. Grevy zebras have narrow black and white striping patterns. A mature one stands between 1.5 to 1.6 metres in height and weighs between 350 to 450 kilograms. The population size of Grevy's zebra has been declining drastically despite conservation efforts, such as the hunting ban by the Kenyan government in 1977. However, the role of disease causing parasites has not been adequately addressed. This study was conducted with the aim of identifying the various types of gastro-intestinal (GIT) parasites in Grevy's Zebras in the Samburu landscape of the Samburu County in the Northern Kenya. It also targeted to determine parasitic infestation levels within the different age categories and sex of the animals. Age determination was done through observation of their height, body coloration and mane length at their back. This study was conducted between July and December 2010. Fresh dung samples were collected from the field and observed for parasites in the laboratory using a light microscope. Parasites were identified by use of qualitative methods including direct saline preparation, formol-ether sedimentation technique and Haranda Mori Culture techniques. Quantitative method that uses Cornell-McMaster dilution egg counting technique was used to determine the parasite infestation level in each faecal sample. In total, nine (9) types of parasites were identified from a total of 207 faecal samples that were analyzed. This included both the helminthic and the protozoan type. The protozoan type recovered included, the *Eimeria leuckarti* (15%), *Entamoeba equi* (13%), *Balantidium coli* (1.0%) and *Giardia intestinalis*. (1.0%) while the helminthic type included *Strongylus vulgaris* (98.1%), *Strongyloide westeri* (55.6%), *Trichonema sp* (5.8%), *Parascaris equorum* (5.3%), and *Trichuris trichiura* (0.5%). All the samples (100%) were found to harbour single or mixed type of helminthes and protozoan parasites. *Strongylus vulgaris* had the highest occurrence while the lowest was recorded by *Trichuris trichiura* in the helminthic group while in the protozoan type, the highest was *Eimeria leuckarti* while the lowest was *Giardia intestinalis*. KruskalWallis Test was used to compare infestation levels within the three zebra age categories. The age categories included the foals, the juveniles and the adult. The test revealed that there was a significant difference in the number of parasites in the three age categories ($H = 6.522, P < 0.05$). The parasitic infestation level was higher in adult grevy zebras than in foals and juveniles. Analysis using Mann-Whitney test was used to determine infestation levels between males and females. This test showed that there was no significant difference in infections between males and females ($U_{(16)} = 40, P > 0.05$). In order to prevent the spread of gastro-intestinal parasites, the contamination of pasture land should be prevented by treating the hosts with antihelminthics. In this regard, the Samburu pastoralists should be encouraged to use antihelminthics to treat their animals since they share similar habitat with the Grevy zebras and this will reduce cases of cross transmission of the parasites. The government or non-profitable development agencies should include Grevy zebras in their priority lists of research and develop sustainable integrated diseases prevention and control programs that are practical for developing communities.

CHAPTER ONE: INTRODUCTION

1.1 Background Information

Grevy's zebra is the common name for the largest species of zebra and its scientific name is *Equus grevyi*. It is characterized by large, rounded ears, erect mane, and a coat with narrow and close-set black and white stripes that extend to the hooves. Grevy's zebra, also known as the Imperial zebra, is the largest wild member of the horse family, Equidae. It was used by the ancient Romans in circuses (Prothero, 2003). Currently, their main value is tourism whether in the field or in zoos. This species occurs in northern Kenya specifically in Samburu region of the Samburu County.

The species once ranged over large tracts of south western Somalia and northern Kenya, as well as southern Ethiopia, with 93% of the population occurring within Kenya. The distribution range of Grevy's zebra has dramatically reduced in size. Today, the distribution of the Grevy's Zebra is restricted to Kenya, Ethiopia and Southern Sudan (IUCN, 2010). In Kenya the largest subpopulation is found Samburu which is estimated at 255 individuals (IUCN, 2010). This is a decline from a previous estimate of between 1,838 and 2,319 (Mwasi and Mwangi, 2007). This suggests a marginal increase in Grevy's zebra numbers from the previous estimate of the year 2004. A more comprehensive survey undertaken in 2008, gave an estimated national population of 2407 (Low *et al.*, 2008). Another survey similar to the one conducted in 2008 was done in 2012 but covering a larger area and the use of light aircraft estimated the national population at 1897 animals. The latter study reported 510 fewer animals than were detected in 2008. In these two surveys most sightings (89%) were in the Wamba (Samburu) and Laikipia areas of Kenya.

From the foregoing discussion, it is clear that the Grevy zebra had been declining both in its distribution range and in numbers in recent times (Rowen *et al.*, 1992). In the Samburu region,

there are unconfirmed reports that there could be subsistence poaching in this region yet little attention has been given to their population. One possible cause of decline in any wild animal population is diseases due to parasitic infections. The likely impact of disease on the Grevy's Zebras population was illustrated by a recent assessment following an outbreak of anthrax during 2005 – 2006 in Wamba, Samburu County, which killed *E. grevyi*, plain zebras, and domestic donkeys (Belinda *et al.*, 2009). The affected *E. grevyi*, were the foals and adults while the juveniles were less affected. The already small population of the species was at stake because the juveniles are dependent on the grown individuals, particularly adult females, for food (in form of milk), guidance and security. Thus, the impact of disease on the *E. grevyi* population may therefore be of higher significance than is currently thought. Despite this risk, there is no information on the type gastrointestinal parasite that affect the Grevy zebras in the Samburu region and the age categories most vulnerable. Therefore, the current study designed to investigate the gastrointestinal parasites harbored by the zebras and the age categories affected. This study looked at Gastro Intestinal Tract (GIT) parasites as indicators of the general health of *E. grevyi*. The aim was to identify the zebra age and sex categories particularly affected by the parasites and make recommendations on the conservation of the species. The age categories included foals, juveniles and adults.

1.2 Problem statement

There is very little information on disease and epidemiology in these free ranging Grevy zebras. Since the 1970s there has been a steady decline in the Grevy's Zebra's numbers (IUCN, 2010) leading to the current low numbers. In Kenya, the Samburu subpopulation of Grevy's Zebra is the largest and probably the most stable. Despite the Kenyan government putting in place a number of protective measures including the enactment of a ban on hunting in 1977 and current efforts to uplift the species from a game animal to a protected one, the species numbers have not recovered (IUCN,2010). However, there has been some marked

increase in the population, particularly in areas where competition for resources, water and food, with domestic animals has been reduced as observed in Laikipia Plateau (Williams, 2002) and generally on private land. The coexistence of domesticated animals and the grevyi zebras brings a possibility of cross transmission of parasites. *Strongyloids sp* is one of the examples of parasites that is known to infect both the domesticated animals and the grevyi zebras (Regassa *et al.*, 2006).

1.3 Justification

It is not well understood why the *E. grevyi* population has failed to increase given the conservation and management measures in place like hunting ban, integration into ranches and revision of protection status. Parasites lead to reduced reproductive success due to illnesses associated with the parasites. However, neither the diversity of parasites nor the levels of parasites infestation affecting their health is known (Radcliffe and Osofsky, 2002). It is therefore important to identify the gastro intestinal parasites and their infestation levels among the Grevy's zebras. Monitoring *E. grevyi* numbers and habitat condition as well as ascertaining parasite and diseases surveillance is important in maintaining adequate numbers of healthy animals. Parasitic infection decreases the production and productivity in the animals, mainly as reduction of body weight, failure to gain weight or mortality in acute cases. It is therefore important to conduct a study on parasitic infections for these Grevy's zebras in Samburu landscape as this would inform Grevy's zebra management and other stakeholders on the threats of the parasites and hence plan for conservation strategies.

1.4 Research Questions

- i. What is the species composition of gastro intestinal tract parasites infecting in *E. grevyi* zebras of the Samburu landscape in Samburu County?
- ii. What is the relative abundance of the gastro intestinal parasites in the different age categories of the *E. grevyi* zebras of the Samburu landscape?

- iii. How does gastro intestinal tract parasite infestation level vary with sex categories in *E. grevyi* zebras?

1.5 Hypotheses

- i. There are no gastro intestinal tract parasites species infecting *E. grevyi* zebras of the Samburu landscape in Samburu County.
- ii. There is no difference in relative abundance for the gastro intestinal tract parasites infecting the different age categories of *E. grevyi* zebras of the Samburu landscape.
- iii. Gastro intestinal parasites infestation levels are not the same among the sex categories of *E. grevyi*.

1.6 General Objective

To assess the infestation levels of gastro intestinal parasite in different age and sex categories of the *E. grevyi*.

1.7 Specific Objectives

- i. To identify the various species composition of the gastro intestinal parasites that infests the *E. grevyi* population in Samburu landscape.
- ii. To compare the relative abundance of the gastro intestinal tract parasites infecting different age categories *E. grevyi* zebras of the Samburu landscape.
- iii. To determine the gastro intestinal parasite infestation levels between males and females (sex) among the *E. grevyi*.

1.8 Significance of the study

The work specifically investigated the different forms of parasites infesting the animals and the level of infestation with age and sex. This study revealed new information on *E. grevyi*'s health in the wild which could be shared with policy makers involved in the conservation of *E. grevyi*. This work revealed that these animals are infested by various forms of parasites mainly of the helminth and the protozoan types. The adults were found to have a higher

parasitic infestation level compared to the juveniles and the foals. For this reason any control programme should target the adults if it is to succeed. The study was conducted in community conservancies where the animals share a similar habitat with the domesticated animals giving them a higher possibility of cross transmission of these GIT parasites. In this case if the animals are to be safe, a policy could be formulated to isolate the grevyi zebras from the domesticated animals. These results provide information to conservation organizations involved in management of GIT parasites. The findings are therefore of importance to livestock stakeholders since it gives them the proper information enabling them to determine the necessary intervention measures. This outcome can be used by the conservation organizations and others in place to effect species translocation to areas where the equine failed such as in Djibouti and Somalia and this would enhance their diversity.

CHAPTER TWO: LITERATURE REVIEW

2.1 Conservation status of Grevy's Zebra

The *E. grevyi* low numbers remain a conservation mystery, especially with the depopulation drivers kept at a favorable minimum (Earthwatch, 2010). *E. grevyi* is on the brink of extermination in Samburu (Kenyan stronghold) whereas in Somalia and Djibouti it is extinct (IUCN, 2010). Their numbers and range have declined dramatically over the last three decades (Williams and Low, 2004). In the past, this was due to hunting for its skin that fetched high prices on the world market. Currently, it suffers from habitat destruction, human disturbance at water holes and competition with domestic grazing animals (AWF, 2010). Historically, the species ranged into the Far East where it is also extinct (Earthwatch, 2010). The loss of this kind is consistent with that of its other relatives making it the only living representative of subgenus *Dolichippus* (http://en.wikipedia.org/wiki/Grevy's_Zebra) which flags the need for its preservation. Fossils of zebra-like equids have been found throughout Africa and Asia in the Pliocene and Pleistocene deposits. Notable examples included *E. sanmeniensis* from China, *E. cautleyi* from India, *E. valeriani* from central Asia and *E. oldowayensis* from East Africa. The latter, in particular was very similar to the Grévy's zebra and may have been its ancestor (Prothero, 2003).

2.2 Parasitic infections as a conservation concern

Ideally *E. grevyi* exhibits social organization (Cordingley *et al.*, 2009) and individuals of the different age category would occur and defecate more or less homogeneously with equal chance of finding samples from whatever age group. The close association of *E. grevyi* and livestock on the Samburu pastoral lands creates favorable conditions for cross transmission of parasites and diseases. Further, veterinary services at the remote study site are limited both for livestock as well as for *E. grevyi* and other wild animals. Parasitic and disease transmissions

to the *E. grevyi* therefore remain in the population leaving individuals struggling with the parasite load. Generally ungulates (mammals with hooves) in which the *E. grevyi* is included are affected by various forms of endoparasites. Such parasites include *Strongyloides sp.* and *Trichostrongylus sp.* which infect the deer (*Capreolus capreolus*). *Trichostrongylus sp.* which infect the *Equus sp.* (Pony Horse). *Nematodirus sp.* which infects the *Taurotragus oryx* (Eland). The *Eimeria sp.* and *Nemethodirus sp.* which infect *Bos indicus* (Zebu). The *Trichuris sp.* which infects *Camelus dromedarius* (Camel). The *Trichuris sp.* also infects *Ovis sp.* (Sheep) (Atanaskova *et al.*, 2011).

Indeed a study done in Oromia, Ethiopia, an area with similar ecological conditions like the Samburu ecosystem, revealed that *Strongylus sp* and *Eimeria sp* were the most prevalent parasites in domestic cattle, sheep and goats (Regassa *et al.*, 2006). The three primary species of strongyles that infect the equids are *Strongylus vulgaris*, *Strongylus endentatus*, and *Strongylus equinus*. In a study of parasites in captive herbivores derived from the wild including equids, *Strongylus*, *Trichuris*, *Eimeria* and amphistomes were most prevalent (Singh *et al.*, 2006). Amphistomes are snail-borne trematodes that infect rumens and reticulums and are capable of causing acute and chronic disease in ungulates. The main endoparasites that infect equines are *Ascaris equorum*, *Parascari sp.*, *Strongylus sp*, *Trichonema sp.*, *Triodontophorus tenicolluis*, *Anoplocephala spp.*, *Gastrodiscus aegyptiacus*, *Strongyloides westeri*, *Dictyocaulus arnfieldi*, and *Oxyuris equi*, (Soulsby, 1978). Threadworm occurs mainly in foals especially those still suckling. Other Infections come from dams, upon drinking the infected water, causing debilitating diarrhea. Roundworms especially in foals tend not to damage the gut but compete for food leading to poor development. Severe infestation can cause dangerous colic. Parasite eggs are viable in soil for a very long time. The equine lungworm, *Dictyocaulus arnfieldi*, has a worldwide distribution, particularly in areas with heavy rainfall. Donkeys are a common host and may, in some areas of the world, be the

reservoir of infection for horses. The parasite is not harmful in low numbers but large numbers can cause death.

In captive wild animals, GIT parasites have been shown to cause reduced fertility (Regassa *et al.*, 2006), increased mortality and morbidity (Rao and Acharjyo, 1984). Mortality refers to the incidence of death or the number of deaths in a population while morbidity refers to an incidence of ill health in a population. A heavy internal parasite burden affects the health of the animal adversely, particularly when they are undernourished and stressed. Nematodes cause physical damage, ranging from mild digestive upset and lower feed absorption, to severe colic (all forms of gastrointestinal conditions which cause pain), due to intestinal blockage or intestinal rupture. Signs of roundworm infection include fatigue, potbelly, rough hair coat, and slow growth. Young horses develop nasal discharge accompanied by a cough. The worms are the most common parasites of the Grevy's Zebras and they can cause considerable morbidity and mortality (Herd, 1990). Their presence for example is shown by larval cyathostominosis (previously cyathostomosis or cyathostomiasis), a syndrome in which large numbers of larvae emerge from the walls of the large intestine and caecum and cause severe colitis that may result in death (Mair, 1994, Van Loon *et al.*, 1995). Cyathostominosis is caused by the Cyathostomin parasites which are also known as small strongyles. Gastrointestinal tract (GIT) parasites are widespread in Tanzania (Makundi, 2001, Keyyu *et al.*, 2002) and limit cattle production in many areas and countries. Similar to other equines, zebras feed with their lips along the soil surface and prefer areas with short grass (Moelhman, 2003). This foraging strategy of short grasses and grazing closer to the ground exposes them to a higher risk to contract infection through ingesting soil containing parasitic infections.

Young and malnourished animals are most susceptible to the parasites eggs and larvae. Various types of worms are transmitted when an animal eats grass or drinks water

contaminated with larvae or eggs. The larvae are very resistant to harsh environmental conditions because of a protective sheath. The problem is especially common in the rainy season (IIRR, 1996). The parasites cause direct or indirect losses of lives of the infected animals. Direct losses are attributed to acute illness and death. Indirect losses include the diminution of productive potential, such as decreased growth rate, weight loss in young animals and late maturity (Hansen and Perry, 1994).

Nematodes presences in equines were reported (Fisher *et al.*, 1992, Karki and Manandhar, 2006) while working on occurrence of helminthes parasites in mules in Nepal. The study documented associations between the acute sudden death of mules with colic symptom and parasites burden. It is therefore likely that competition optimizes conditions for parasitic and disease infections in the *E. grevyi* resulting in low recruitment and survival rates which has led to the observed low population.

Besides nematodes, protozoans such as *Entamoeba coli* and *Balantidium coli* have been found to cause problems in animals. *Balantidium coli* and *Entamoeba histolytica* cause balantidial and amoebic dysentery respectively both of which are zoonotic water and food borne infections. There is an association between parasitic infection and the quality of drinking water (Roche *et al.*, 1999). *Entamoeba coli* are also an important diarrhoeal pathogen in man (Okeke *et al.*, 2003). *Entamoeba sp* and *Giardia sp* are potentially pathogenic and in many parts of the world either or both organisms constitute a health problem (WHO, 1980). Infection is distributed worldwide with high occurrence in tropical countries where the presence of warm environment creates favorable conditions for parasite development particularly the gastrointestinal parasites.

Studies of the gastrointestinal tract of horses revealed that it provides a target site for many internal parasites species, such as *Parascaris equorum*, *Habronema spp.*, *Gasterophilus spp.*,

and tapeworms. The parasites have the potential to cause serious disease conditions in horses including diarrhea, emaciation, colic, anaemia, haemorrhage and death (Mfitlodze and Hutchinson, 1989). Parasitic helminthes have been reported to be one of the most common factors that constrain the health and working performance of donkeys worldwide (Githiori *et al.*, 2004). Parasites cause various degrees of damage depending on the species and number present, nutritional and the immune status of equids. In donkeys, infection by endoparasites are responsible for problems including poor body condition, reduced power output, diarrhea, colic, emaciation, impaired growth, poor reproductive performance, short lifespan and predisposition to other infectious diseases (Fikru *et al.*, 2005; Yoseph *et al.*, 2005; Ayele *et al.*, 2006; Getachew *et al.*, 2009, 2010). Studies on GIT parasites in working donkeys (ones doing domestic activities like water fetching and fire wood collection) across several countries of the world have disclosed the involvement of several common parasite species which included *Strongylus sp.* and *Strongyloide sp.* (Sotiraki *et al.*, 1997; Wells *et al.*, 1998; Mushi *et al.*, 2003; Pereira and Vianna, 2006; Uslu and Guclu, 2007). The investigations have revealed that in developing countries where nutrition and hygiene are generally poor, soil transmitted helminthes (STH) are highly prevalent and hence a major problems of equids. *Ascaris sp.* and *Trichuris sp.* are examples of parasites that are soil transmitted (Crompton, 1999).

2.3 Life cycles of some of the parasites

Most of the parasite life cycles start out as an egg. The egg matures into larvae. The larvae mature into an adult that lays eggs. Internal parasites spend a big proportion of their life cycle inside equine's stomach or intestines. The eggs or larvae are deposited onto the ground by an infected animal which are then swallowed by other animals as they graze in the pasture. These juvenile parasites then mature into egg-laying adults in the gastrointestinal system. In some worm species, the larvae migrate into other organs, such as the lungs or liver to mature before

returning to the intestines as adults to lay eggs. The eggs are then passed from the animal into the pasture where either reinfection occurs or another animal consumes them to start the life cycle over again. The common parasites are strongyles, roundworms, pinworms, lungworms, tapeworms, and more. In most areas in the tropics, animals continuously graze on pasture all year round. This exposes the animals to continuous parasite pressure when climatic conditions are favorable for the development and survival of free living stages of the parasites. It has been demonstrated that the dominant species, of the endoparasites are found in semi-arid areas (Gatongi *et al.*, 2003). Such dry areas are related with the conditions of the current study area of the Samburu landscape.

2.3.1 Helminthes - Large Strongyles (blood worms, red worms)

They start out as eggs passed in the faeces which hatch into larvae that are consumed by equines as they graze or drink from infected water or food. The larvae mature in the intestinal tract. One type (*Strongylus vulgaris* or bloodworm) migrates into the blood vessels of the small intestines (Fig.2.1). Their entire life cycle takes about 6 to 7 months. The other two types (*Strongylus edentatus* and *S. equines*) migrate into the liver. Their entire life cycle takes about 8 to 11 months. Heavy bloodworm infestation can cause severe or even fatal colic or blood vessel ruptures that lead to extensive blood loss (Mair, 1994).

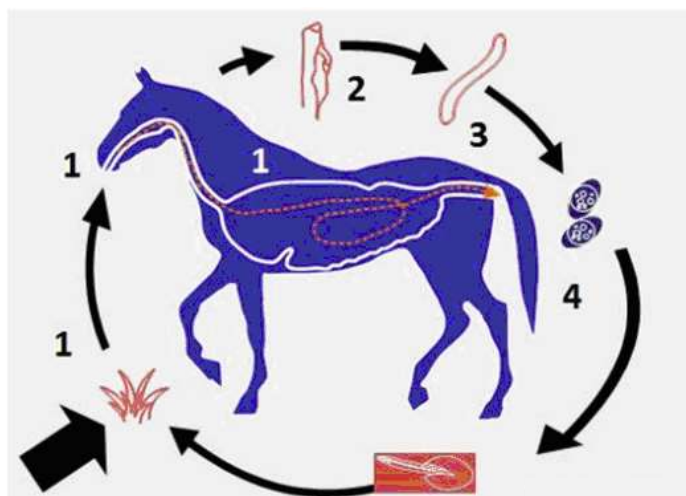


Fig. 2.1: A typical strongyle life cycle (R. C. Krecek, 2004)

2.3.2 The life cycle of ascarids (roundworms)

It resembles that of the strongyles but the infective stage is a very thick-walled egg, which can live in the environment for up to 10 years. Infective larvae stages are ingested while the animal is grazing, and migrate to the large intestine and then to other organs (Fig. 2.2). These include the liver, pancreas and arteries, which supply blood to the intestine. This migration has been implicated as a cause of colic and death in equines. These worms are large, reaching a length of 28–50 cm. These worms can occur in large numbers, which can cause obstruction of th

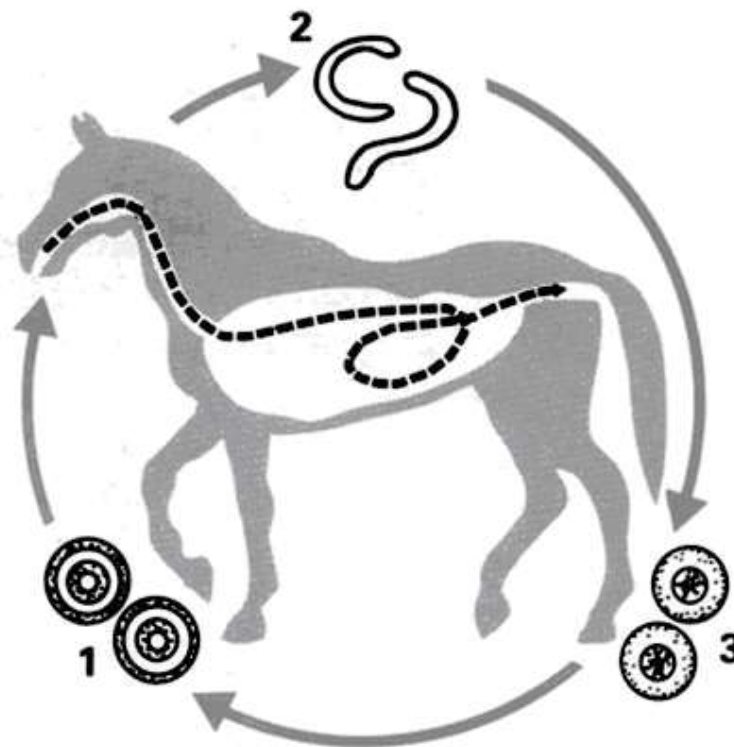


Fig. 2.2: An ascarid life cycle (Pittman – Moore, 1982)

2.3.3 The life cycle of the threadworms (*Strongyloides*).

The life cycle begins particularly when larvae are ingested by the foals from the mare's milk or larvae present in the bedding. They then penetrate the foal's skin and later migrate through the lungs and small intestine. The life cycle takes only about 2 weeks and their main effect is diarrhea.

2.3.4 Life cycles of protozoan parasites

Four main groups of protozoa are recognized on the basis of their locomotion using specialized subcellular and cytoskeletal features, they include the amoebae like *Entamoeba sp*, the ciliates like *giardia sp*, the flagellates and the sporozoa. Most protozoa have enormous reproductive potential because they have short generation times, undergo rapid sequential development and produce large numbers of progeny by asexual or sexual processes. These characteristics are responsible for many protozoan infections rapidly causing acute disease syndromes. Parasites may multiply by asexual division (fission or budding) or sexual reproduction (formation of gametes and fertilization to form zygote, or unique process of conjugation where ciliates exchange micronuclei). Protozoan developmental stages occurring within hosts generally consist of feeding trophozoites, and they may be found within host cells or in hollow organs, body fluids or interstitial spaces between cells. While trophozoites are ideally suited to their parasitic mode of existence, they are not very resistant to external environmental conditions and do not survive long outside of their hosts. To move from host-to-host, protozoan parasites use one of four main modes of transmission: direct, faecal-oral, vector-borne and predator-prey transmission. The one that is directly related to the study is faecal-oral transmission (Figure 2 3).

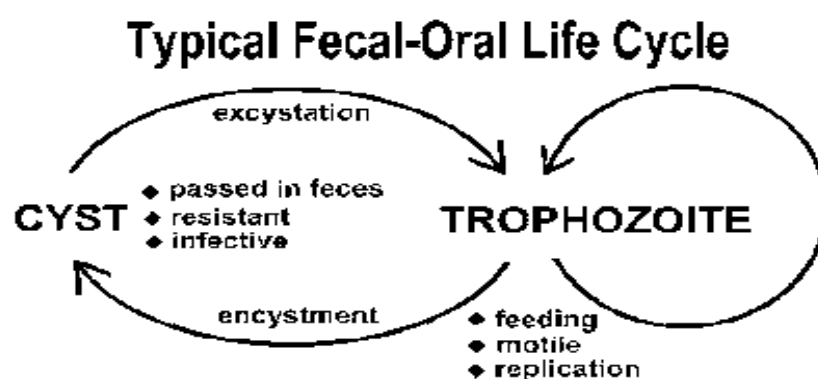


Figure. 2.3: Protozoan parasite life cycle (Mark F. W., 1999)

In this method of faecal-oral transmission, environmentally-resistant cyst stages are passed in faeces of one host and ingested with food or water by another (e.g. *Entamoeba sp.*, *Giardia sp.*, and *Balantidium sp.*). The parasites form faecal cysts which are ingested by new hosts leading to new infections.

2.4 Gastrointestinal protozoan parasites in equines

The equines provide shelter and subsistence to different kinds of protozoan parasites which live, as internal internally. Protozoan parasites are grouped into flagellates, ciliates, amoebae and apicomplexa. Flagellates possess flagella at some life stage and they include the *Giardia sp.*, *Hexamita sp.*, *Histomonas sp.*, *Trichomonas sp.*, *Trypanosoma sp.* and *Leishmania sp.* Ciliates on the other hand possess cilia at some life stage and they include the *Balantidium sp.* Amoebae group use pseudopodia for locomotion at some life stage and this is shown by the *Entamoeba sp.*, and *Naegleria sp.* Apicomplexa includes the *Eimeria sp.*, and the *Plasmodium sp.*. Trypanosomes are the blood-inhabiting parasite. Fortunately, several of the more pathogenic, or disease-producing species, are limited in their distribution and incidence. The most numerous of these parasites are the ciliated forms that live chiefly in the colon. Except for a few species, the protozoa of equines fall into two groups, those that inhabit the blood and those that live in the large bowel. They can be detected readily by microscopic examination of a watered drop of fresh manure. The trypanosomes are by far the most important protozoan parasites of equines. They cause such diseases as nagana, which takes a heavy loss among the equine populations of regions where it has become established. The disease nagana is prevalent in east and central Africa. The commonest agent of the infection is *Trypanosoma brucei*, a variety which is the cause of sleeping sickness of man. In equines the disease is

usually fatal in a few weeks. Ciliated intestinal protozoa belonging to many genera are common inhabitants of the large bowel of equines. Study on parasitic infections of ungulates in Ethiopia reported them to harbor intestinal protozoans including *Cryptosporidium spp.*, *Giardia lamblia*, *Balantidium coli* and *Eimeria sp.*, (Uysal *et al.*, 2009). Among other intestinal protozoans, *Cryptosporidium sp.* has been given much attention because of its occurrence in various domestic and wild animals and its potential for causing cryptosporidiosis (Ramirez *et al.*, 2004). This condition causes severe diarrhea and weight loss in animals.

2.5 Gastrointestinal helminthes infecting equines

There are various types of helminth parasites infecting the equines. The main groups are the large strongyles, small strongyles, ascarids and tapeworms (Nielsen *et al.*, 2010). Large strongyles, known also as blood or red worms, enter the horse via ingestion of infective larvae from feces. After emerging in the small intestine they migrate through body tissues before returning to the large intestine to mature. They cause significant damage to body tissues. Adult strongyles are blood feeders and can cause extensive damage to the intestine wall that can result in anemia, as well as weight loss and diarrhea (Andersen *et al.*, 2013).

On the other hand, small strongyles, or cyathostomins, differ from their larger counterparts in that they remain within the intestinal tract. They develop in the gut wall and emerge as larvae. This emergence may cause slight damage to the intestinal wall but cyathostomins are less severe in their effects than the blood worms. However, if there is mass emergence of these larvae then significant damage may occur. This situation is known as larval cyathostominosis (Nielsen, 2010). Ascarids mainly infect young animals via ingestion from contaminated pasture, the most notable of which is *Parascaris equorum* (large roundworm). This parasite migrates through the liver and lungs before returning to the small intestine. In severe challenges, respiratory problems could also be observed (Andersen *et al.*, 2013). Tapeworms

(cestodes) are also found in the intestines. In some cases, there are no clinical signs with mild infections but anemia, weight loss, ulceration and digestive aberrations could result from more severe challenges (Andersen *et al.*, 2013).

Helminths feed on living hosts. They receive nourishment and protection while disrupting their hosts' nutrient absorption, causing weakness and diseases. They can live inside equines and other animals. Helminths are able to survive in their hosts for many years due to their ability to manipulate the immune response by secreting immuno-modulatory products (Jirillo *et al.*, 2014). Helminth ova (or eggs) have a strong shell that protects the eggs against a wide range of environmental conditions. Equines are hosts to great nematodes of the family Strongylidae. The most common gastro intestinal nematode parasites of equines include large strongyles (*Strongyloide sp.*), small strongyle (*Strongylus sp.*), the round worm (*Ascaris sp.*) and the pinworms (*Oxyuris equi*) (Zerihun *et al.*, 2011).

Helminthes parasites, particularly strongyle nematodes are the common inhabitants of the gastro-intestinal tract of equines and can cause infections with clinical signs ranging from ill-health to sudden death (Umur and Acici, 2009). All equids including zebras host a fascinating biodiversity of parasites that inhabit the organs and tissues. Strongyles are one of the most common and by far the most diverse parasitic group comprising many species, (Krecek, 1994). The zebras also host more than 100 million small pinworms per individual animal and show no signs of pathology or clinical disease. Pinworms are common in horses causing perineal irritation which leads to rubbing the tail head which results in broken hairs.

2.6 Relationship between age and parasitic infection in herbivores

Herbivores generally acquire gastrointestinal parasites by ingesting spores, eggs or larvae together with food and water (Regoes *et al.*, 2003). Parasitic studies revealed that the prevalence of small Strongylids significantly increased with age (Collobert *et al.*, 1995). Ascarids are common in horse foals and yearlings but due to immunity do not occur in horses

after a year of age. In contrast, ascarids occur in young donkeys and continue to occur in older ones. It was observed that Ascarids mainly infect young animals via ingestion from contaminated pasture and the most notable of which is *Parascaris equorum* (large roundworm) (Andersen *et al.*, 2010).

Threadworms affect primarily the foals. Fecal examinations seldom reveal threadworm eggs in adult horses. Foals may acquire the infection through larvae present in the mare's milk. Threadworm larvae are found in mare's milk from 4 to 40 days after foaling and foals may become severely infected by two to three weeks of age, exhibiting diarrhea, indigestion, and unthriftiness (Radostits *et al.*, 2007) on parasitic studies reported that young animals do not have well organized immune system and this result in them having a higher chance of parasitism than the older equines.

2.7 Relationship between sex and parasitic infection in herbivores

Studies in different regions have revealed some relationship between parasitic infections and sex (Mfitilodze and Hutchinson, 1990) on a parasitic study in equines found that male horses had lower overall prevalence of Strongyles compared to females. In another study it was found that *Parascaris equorum* in mares (females) higher than stallions (males) (Kornas *et al.*, 2007). A study on equine foraging behaviour revealed that lactating females' take significantly more bites per step than males. This was also observed in plain zebras within Etosha National Park in Namibia (Neuhaus and Ruckstuhl, 2002). Grevy's zebras studies revealed that lactating female use more areas with greener but shorter grass more often than both bachelor groups and this exposed them to high risks to parasitic infections compared to males (Sundaresan *et al.*, 2007). Specific nutrient demands of lactation may drive lactating females to have a higher bite rate and this increases the chances for the females to ingest the parasites. Prolonged drought in equine habitats cause wilting of vegetation forcing the herbivores to graze closer to soil that is potentially infected with parasites (Dragon and

Rennie, 1995). The foraging behavior play a critical role in influencing the contact with the pathogen in the ground and establishment of the infection. Herbivores increase their foraging efficiency to acquire sufficient nutrients at times when nutrient quality is high. Zebras are nonselective grazers (Moelhman, 2003), they feed a lot but since the females have a higher bite frequency, this exposes them to high risks of parasitic infections. Factors like age, sex, breed, physiological status of the host affect the intensity and prevalence of infection of parasites in horses, which can be more serious in young and undernourished horses and mares (Lyons *et al.*, 2012).

CHAPTER THREE: STUDY AREA, MATERIALS AND METHODS

3.1 Study area

This study was conducted in Wamba Division of Samburu County in the northern part of Kenya (Figure 3.1).

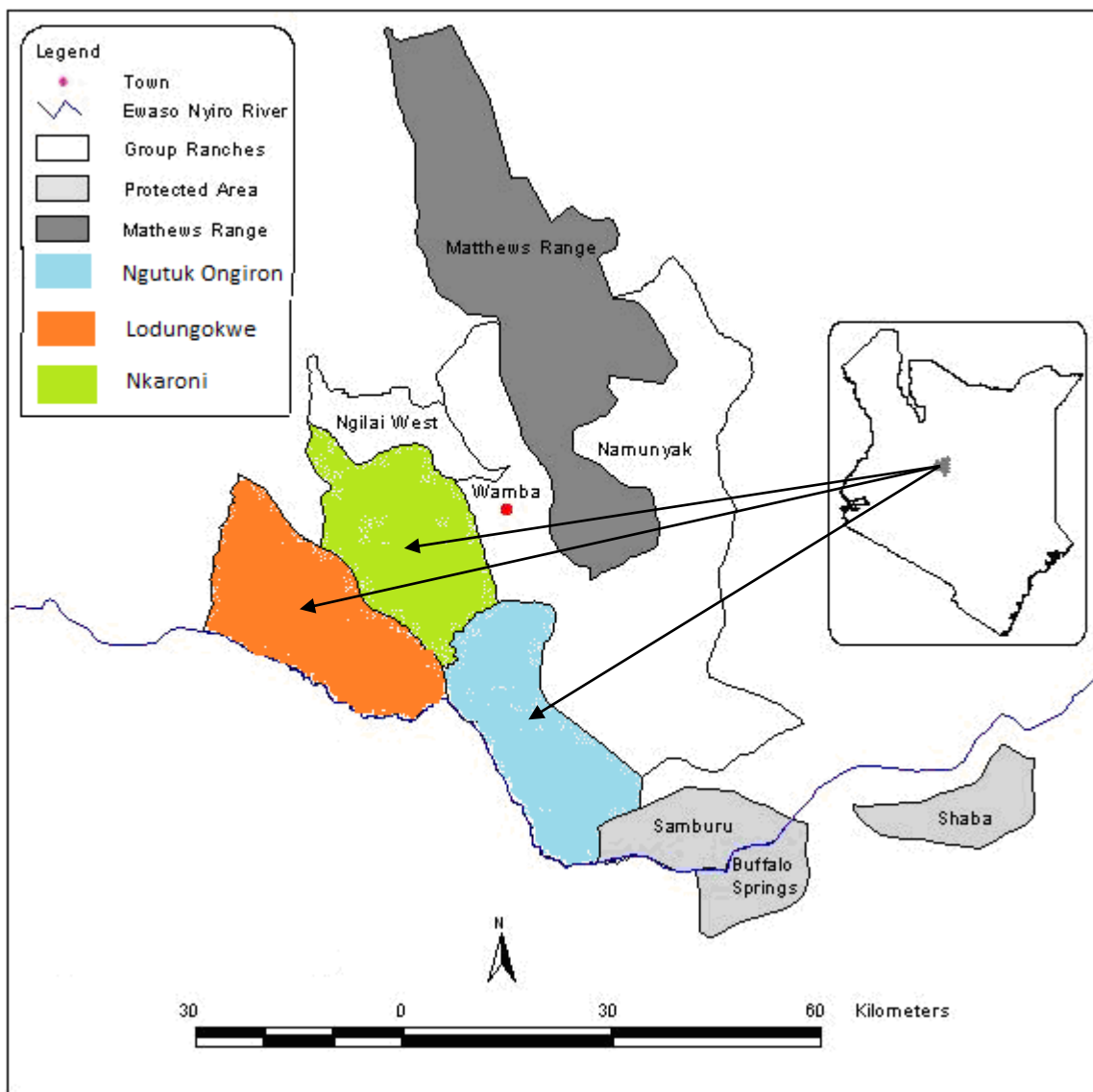


Figure 3.1: Map of the study area

The study area lies to the northern Kenya at about 37°E and 1°N. It borders the Samburu National Reserve to the south and the foothills of Matthew Ranges to the north. This is a hot

and dry area with low, erratic rainfall (Pratt and Gwynne, 1971). Mean annual rainfall is 375mm whereas the mean minimum and maximum temperatures are 24°C and 33°C respectively. The area is rich in wildlife species including elephants (*Loxodonta africana*), Grevy's zebras, plain zebras (*Equus quagga*), reticulated giraffes (*Giraffa camelopardalis reticulata*), Grants gazelles (*Nanger granti*), gerenuks (*Litocranius walleri*), dik diks (*Madoqua kirkii*), Beisa oryx (*Oryx beisa beisa*), bat eared fox (*Otocyon megalotis*), wild dogs (*Lycaon pictus*), lions (*Panthera leo*), hyenas (*Crocuta crocuta*), cheetahs (*Acinonyx jubatus*), pancake tortoises (*Malacochersus tornieri*), shrews (*Sorex araneus*), rodents, and various birds including the Somali ostrich (*Struthio camelus molybdophanes*), vultures (*Aegyptius monachus*) and helmeted guinea fowls (*Numida meleagris*). Land is owned communally by Samburu pastoralists and is subdivided into conservancies owned by clans. The sampling sites were Westgate (Ngutuk ongiron), Nkaroni and Barsalinga (Iodungokwe) conservancies (Fig. 3.1). Livestock reared in the area include cattle, goats, sheep, donkeys and camels. This area is very important for Grevy's zebra conservation since it provides a suitable habitat for these animals.

The Samburu people move from place to place with their herds of cattle, camels, goats, and sheep. Unfortunately, the Samburu way of life is being threatened by the impacts of climate change. According to Kenya's National Drought Management Authority, drought in Samburu County leads to conflict, human and livestock displacement, livestock disease, and food insecurity. According to the United Nations Environment Program (UNEP), the Samburu keep the indigenous breeds of small East African Zebu Cattle, Red Maasai sheep, and East African goats that are highly adapted to the local environment due to evolution and selective breeding. Because they are well adjusted to local conditions, indigenous breeds are more successful than exotic breeds in times of drought and disease, and therefore have a significant impact on the survival of the Samburu people both economically and culturally.

3.3 Study Animals

The study involved parasites from three age categories of Grevy's Zebras: adults, juveniles, foals. The estimation of the three age categories were identified using mainly the external features. The foal category was designated to animals whose ages ranged from birth to one year; juvenile category between one year and four years while the adult category included those above four years. They were distinguished through direct observation of mane length at their back, body size, and body hair brightness, body stripes contrast and how they associated with the others particularly the mares. Foals were characterized by the presence of mane along their back from the head to the tail, with brown, black and white coloration which shortened when they reached adulthood. The juveniles had mane reduction from the tail towards the head and relatively larger than the foal and are found close to their mothers (Plate 3.1). In some cases, the males lived alone in bachelor herds. The adults were characterized by their distinctive black and white stripes and greatly shortened mane length.



Plate 3.1: Adult female and a foal *grevy* zebra



Plate 3.2: Adult male *grevy* zebra

They are also large in size and taller than juveniles (Rowen, 1992). Some adult males (stallions) could be distinguished by living a solitary life and their male organs well evidenced (Plate 3.2), these are the territorial males. Stallions establish territories that average 5.75 km² (Estes, 1991) by marking them with dung piles. Those that are unable to establish territories are free-ranging and they form the bachelors groups.

3.4 Collection of dung samples

The procedure involved searching for Grevy's zebra herds by driving through the study areas with local community scouts as the guides. On encountering a herd of Grevy's Zebras, we ensured that they were not disturbed. Using binoculars they were observed carefully in their natural environment to capture the defecation process in case it happened.



Plate 3.3: Collection of the faecal samples

If it occurred, the age and sex category of the defecating animal was recorded. Immediately after it was dropped, the fresh dung was observed to determine its consistency (whether diarrhea, soft formed or hard formed) and the presence or absence of blood and/or mucus. Then, a small sample was scooped (Plate 3.3) from the fresh dung pile using a wooden spatula (tongue depressor), put in a polypot then labeled and the date and the sample number was recorded on a data sheet (Appendix III). In addition, the coordinates of location of the dung was recorded using a GPS receiver (Plate 3.4).



Plate 3.4: Recording of Sample number and GPS position on a data sheet

To avoid getting samples from the same animal more than once, a herd was not observed for more than three hours since previous observations had shown that an animal is very unlikely to defecate more than once within such a time. Further, the team members were extra attentive when making observations to avoid errors. In this case recording was only done upon the agreement of the team members on the specificity of the animal. In circumstances where confusion came up due to rapid movement of the animals and intermingling, the sample was not collected. The animals were observed for a maximum of three hours since observations had revealed that this was the interval at which an individual defecated. Collected samples were transported to the laboratory in a cool box for analysis. This procedure was repeated on different days.

3.5 Identification of parasites.

To identify the various types of GIT parasites infecting *E grevys* zebras, samples were taken through laboratory procedures. To begin with, fresh faecal samples collected from the field were put in clean labeled sample bottles. Then they were taken to the laboratory for microscopic analysis examination. The methods involved both qualitative and quantitative techniques. Qualitative analysis involved use of formol-ether concentration method, direct saline preparation and haranda mori culture (Urquhart *et al.*, 1996).

3.5.1 Qualitative examination of faecal samples

This method involved direct saline preparation, formol-ether sedimentation method and use of haranda mori culture.

3.5.1.1 Direct saline preparation

A small portion of fresh stool sample was emulsified in physiological saline in clean plastic beaker using a glass rod. A drop of the faecal emulsion was then transferred on to a clean microscopic slide after which a cover slip was placed on top carefully before examination. The wet preparation was observed under a microscope using 10× and then 40× with the

condenser iris diaphragm sufficiently lowered then raised respectively to give a good contrast. Confirmation of various types of parasites was established using a parasite chart which gave the various forms of endoparasites and their shapes. Plates also were used to distinguish between the various forms of endoparasites. This method is useful in examination and identification of trophozoites of protozoa and also for the eggs or larval stages of helminthes (Plate 3.5).



Plate 3.5: Identification of parasites using a microscope

3.5.1.2 Formol-ether sedimentation method

One (1) gram of fresh or formalin-fixed faeces was emulsified in 7ml of 10% formalin then strained (put) into another clean beaker before transferring the strained faecal emulsion into a 15ml centrifuge tube. The final volume was made up to 7ml using 10% formalin before adding 3ml of diethyl ether and shaking the entire suspension vigorously for 1 minute then spinning it at 1500rpm for 10 minutes using a centrifuge. After spinning, the debris at the diethyl ether and 10% formalin inter-phase was dislodged using an applicator stick before discarding the entire supernatant. The pellets were re-suspended in 10% formalin before

transferring a drop on to a clean microscope slide. A cover slip was carefully placed on top after addition of a drop of Lugol's iodine solution. This examination of the wet preparation was done using 10× and then 40× objective lens. This method concentrates eggs/larvae of helminthes and cysts of protozoa in the faecal material for easy detection and identification.

3.5.1.3 Haranda Mori Culture (for some samples) to identify the larval stages

A pea size portion of fresh stool sample was smeared on a clean (Whatman) filter paper No. 1 strip using an applicator stick and then dipped into a clearly labeled centrifuge tube containing distilled water ensuring that the smeared part did not touch the water. The cap of the centrifuge tube was closed loosely to allow for aeration then left at room temperature for 7 to 14 days. The smeared strip was removed from centrifuge tube and discarded safely before spinning the sample at 1500rpm for 10 minutes. The supernatant was discarded and the pellet re-suspended in 0.5ml of 10% formalin. A drop of the pellet was then transferred onto a clean microscope slide before adding a drop of Lugol's iodine. A cover slip was then placed on top carefully before mounting the wet preparation onto a microscope stage and examining the presence of larvae using ×10 and then × 40 magnifications.

3.6 Comparing relative abundance of GIT parasites

To establish a relative abundance of the GIT parasites quantitative method was used.

3.6.1 Procedure

One gram of faecal matter was mixed with 15ml of saturated sodium chloride by stirring using a glass rod. The mixture was strained into another clean beaker. Using a bulb sucker, 0.6ml of the sample was put into two closed Mac-masters chambers. The charged Mac-master slide was then examined under a microscope using 10× and 40× objective lens. Eggs within the grid were counted in the two chambers identifying the genus and the species of different entero-helminthes. The samples determined positive for *strongylus sp* were subjected to McMaster Egg Counting Technique for counting the number of Eggs per gram (EPG) of faeces (Urquhart *et al.*, 1996). Identification of the eggs was made on the basis of their

morphology (Soulsby., 1986). The total number of eggs counted in two chambers was multiplied by 50 (as per the method) to get the number of eggs per gram (EPG) of the faeces;

$EPG = (\text{Number of eggs in first chamber} + \text{Number of eggs in second chamber}) \times 50.$

3.7 Determining the infestation levels of GIT parasites

The Infestation levels of GIT parasites were classified using quantitative techniques. The different age categories of animals which comprised of the foals, juveniles and adults were considered to establish the one with the higher infestation level. This method was used to determine the degree of infection which was categorized as light, moderate and severe (massive) according to the number of parasites eggs count per gram of faeces (EPG). Egg counts from 50-799, 800-1200 and over 1200 eggs per gram of faeces were considered as light, moderate and massive infection, respectively (Soulsby, 1986).

3.8 Data analysis

Data obtained from the faecal samples collected were analyzed and presented using bar graphs and tables of occurrence of GIT parasites in different age categories of the Grevy's Zebras. To compare the infestation level between females and males Mann Whitney test was used. On the other hand for comparison of infestation levels in different age categories Kruskal Wallis test was used. These tests were conducted using PAST (Paleontological Statistics Software package for education and data analysis) version 2.17c to test for any significant difference in parasitic infestation levels among the three age and sex categories of the Grevy's zebra. Non parametric tests were used since the normality of data distribution was questionable.

CHAPTER FOUR: RESULTS

4.1 Socio-demographic characteristics

A total of 207 zebras were sampled throughout the study period. The ratio for male to female is observed to be higher in the juveniles and lowest in adult. Generally the adult grevy zebras were the highest in percentage (70%) followed by the juveniles (22.7%) and the lowest ones were the foals. The number of females was observed to be higher than the males. When considered individually the adult female had the highest number of animals (44%) and the lowest was shown by the female foals (2.9%). The ratio of the male to female was 1:1.25 (Table 4.1).

TABLE 4.1: Sex and age distribution of the sampled Grevy's Zebras in Wamba

	FEMALE(F)	(%)	MALE(M)	(%)	TOTAL	M TO F RATIO	%
ADULT	91	44.0	54	26.0	145	1: 1.69	70.0
JUVENILE	18	8.6	29	14.0	47	1: 0.62	22.7
FOAL	6	2.9	9	4.3	15	1: 0.67	7.3
TOTAL	115		92		207	1: 1.25	100

4.2 Gastrointestinal parasites infesting *E. grevyi* population and their percent (%) occurrences

Examination of faecal samples from a total of 207 Grevy zebras showed the presence of nine types of gastrointestinal parasites. All the samples (100%) were found to harbour single or mixed type of helminthes and protozoan parasite. In total there were 497 parasites recovered of which 435 (88 %) were helminthes while 62 (12 %) were the protozoans.

4.3 Occurrence of Helminthic parasites within fresh dung of Grevy's zebra

Helminthes worms were the most frequent forms of parasites. Specifically, the *Strongylus sp* was the most frequently encountered parasite and was recovered in fresh dung of 203 samples from the total of 207 samples collected. *Trichuris sp* on the other hand was the least frequent and was recovered from the dung of only one zebra. The percentage occurrence of the endoparasites was established based on the number of positive samples of animals found infected by various endoparasites out of the total of 207 Grevy Zebras samples (Table 4.2).

Table 4.2 : Occurrence of Helminthic parasites within fresh dung of Grevy's zebra

Parasite Group	Species	Number of positive cases N = 207	Calculation of the %	% occurrence Of the parasite
Helminthes	<i>Strongylus vulgaris</i>	203	203/207	98.1
	<i>Strongloides westeri.</i>	115	115/207	55.6
	<i>Trichonema sp.</i>	59	59/207	5.8
	<i>.Parascaris equorum</i>	57	57/207	5.3
	<i>Trichuris trichiura</i>	1	1/207	0.5

4.4 Occurrence (%) of different protozoan parasite species in Grevy zebra

The findings in protozoans showed that the highest occurrence was indicated by *Eimeria sp.* (15%) while the lowest was recorded by *Giardia sp.* and *Balantidium sp.* (2 %) each (Table 4.3).

Table 4.3: Occurrence (%) of different protozoan parasite species in Grevy zebra

Parasite Group	Species	Number of positive cases	Calculation of the %	% occurrence
		N = 207		
Protozoans	<i>Eimeria leuckarti</i>	31	31/207	15
	<i>Entamoeba equi</i>	27	27/207	13
	<i>Balantidium coli</i>	2	2/207	1.0
	<i>Giardia intestinalis</i>	2	2/207	1.0

4.5 Photographs of parasites recovered from zebra dung

Pictures of GIT parasites were recovered from the Grevy Zebra dung in the Samburu region taken using a camera mounted on the microscope (Plates 4.1 - 4.10). The parasites show some similarities and differences in morphology and in their classification, for example both *strongyloide* sp and *strongylus* sp originate from phylum *nematoda* but they belong to different families and genera.



Plate. 4.1: Cyst of *Strongyloide sp* (x100)

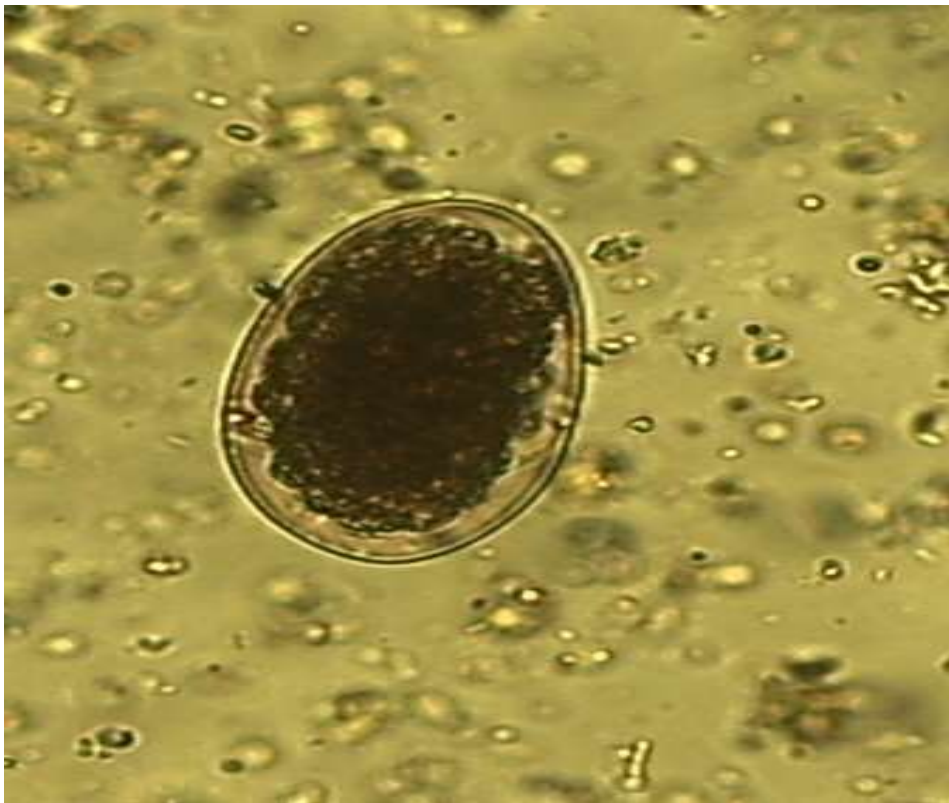


Plate. 4.2: Cyst of *Strongylus sp* (x100)



Plate. 4.3: Larval stage of *Strongyloide sp* (×100)

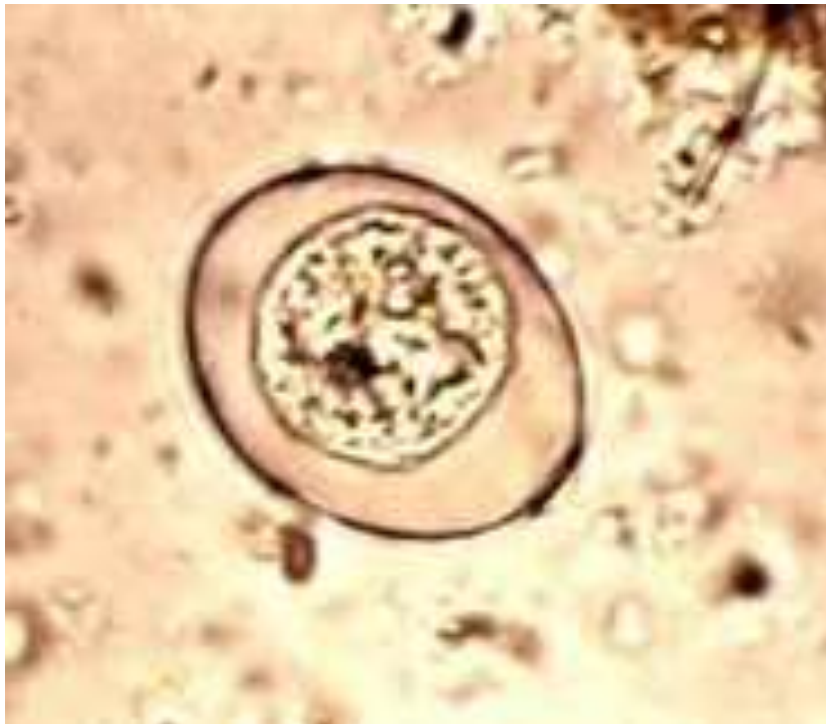


Plate. 4.4: Cyst of *Eimeria sp* (X 100)



Plate. 4.5: Cyst of *Ascaris* sp (X 100)



Plate. 4.6: Cyst of *Trichuris* sp (X 100)



Plate. 4.7: Cyst of *Entamoeba sp* (X 100)



Plate. 4.8: Cyst of *Trichonema sp* (X 100)



Plate. 4.9: Cyst of *Balantidium sp* (X 100)



Plate. 4.10: Cyst of *Giardia sp* (X 100)

4.6 Occurrence of GIT parasites in zebras of various age categories derived through direct smear and sedimentation methods

A total of 497 specimens of parasites were recovered from all the 207 dung samples using direct smear and sedimentation method. *Strongylus sp*, *Strongyloides sp*, *Trichonema sp* and *Eimeria sp* were recovered. *Trichuris sp* were only observed in a single dung sample while *Entamoeba sp* was recovered in adults and juveniles but not in foals. The dung from the adult females harboured seven type of parasite genres with two missing genera which included *Trchuris* and *Giardia* .In adult male both *Balantidium sp* and *Giardia sp*. were missing. The juvenile female had six forms of parasites and three genres were missing. *Trichuris sp* was the only parasite genus that was missing in juvenile males. Both *Entamoeba sp* and *Trichuris sp* were lacking in foals. The foal males also lacked *Balantidium sp* and *Giardia sp*. The genus that showed the highest frequency in occurrence of parasites was the *Strongylus* followed by *Strogyloide* in all the nine types of parasites identified. In terms of scarcity, *Trichuris sp* proved to be the most since it was only recovered once specifically in adult males. When considered age-wise, juveniles had a higher parasite mean recovery (2.56) followed by the adults (2.37) and then the foals (2.17) (Table 4.4).

Table 4.4: Occurrence of various GIT parasites in the different age and sex categories of Grevy's Zebra

Age / Sex of host	Species										
	<i>N</i>	<i>Strongylus</i>	<i>Strongyloid</i>	<i>Ascaris sp</i>	<i>Trichonem</i> <i>a.sp</i>	<i>Trichuris</i> <i>Sp</i>	<i>Eimeria</i> <i>Sp</i>	<i>Entamoeba</i> <i>sp</i>	<i>Balantidiu</i> <i>m.sp</i>	<i>Giardia</i> <i>Sp</i>	Mean parasite recovery
Adult female	91	89	54	24	28	0	15	11	1	0	2.44
Adult male	54	54	24	13	15	1	9	8	0	0	2.30

Juvenile female	18	18	12	5	6	0	2	2	0	0	2.50
Juvenile male	29	28	18	13	7	0	2	6	1	1	2.62
Foal female	6	6	3	1	2	0	2	0	1	1	2.67
Foal male	9	8	4	1	1	0	1	0	0	0	1.67
Total	207	203	115	57	59	1	31	27	2	2	

NB: The Haranda Mori culture method was used only for a few samples since it required a lot of time for the larval stages to be observed. It revealed a few larval stages of *strongyloides sp* which already had been captured through sedimentation and direct saline preparation.

4.7 Parasite load distribution in relation to age and sex of Grevy's zebra

A total load of 8270 gastrointestinal (GIT) parasites was recovered using all the extraction methods when EPG test was conducted from the 207 samples of the study animals. Adult females had a higher parasite load than the males but the difference was not statistically significant. Generally the parasite load increased with increase in age of the Grevy's zebras. In the juveniles there was a relatively higher load in females than males whereas the foals had a slightly higher load in males than females. On average there were 1647, 1301 and 1186 eggs per gram (EPG) in adult, juveniles and foals respectively (Figure 4.1).

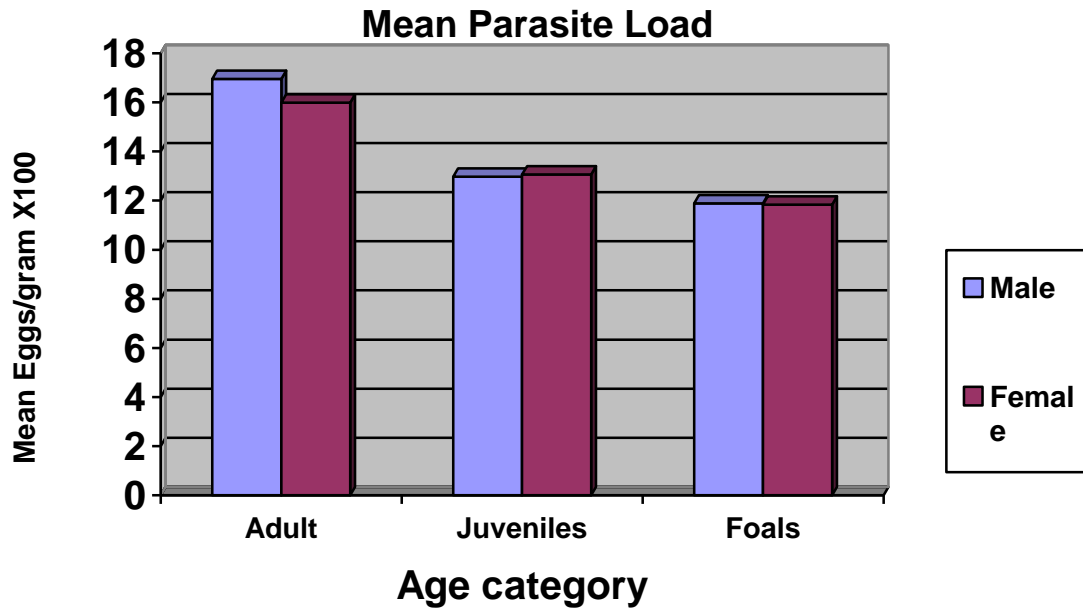


Figure 4.1: Mean Parasite load for different age and sex categories

4.8 Variation in parasite load by age and sex categories

The mean parasite loads for various age and sex categories are shown in Table 4.5. The adult and the juveniles showed severe infection while the foals had moderate infection. This showed that 71.3% suffered severe or massive infection while 28.7% had moderate infection.

Table 4.5: Mean parasite load in Grevy's zebra

Development stage	<i>E. grevyi</i> sex	Level of entero-parasite recovery (Mean EPG x 10 ²)	Average for each age category (Mean EPG x 10 ²)	Degree of infection
Juvenile	Female	13.06	13.000	Severe
	Male	12.97		Severe
Foal	Female	11.83	11.867	Moderate
	Male	11.89		Moderate
Adult	Female	15.99	16.352	Severe
	Male	16.96		Severe

There was a significant difference in the number of parasites in the three categories ($H = 6.522, p < 0.05$). The infestation level was high in adult than the foals and the juveniles. Adult

E. grevyi (mean 16.352) had higher infestation than the foals (11.867) and the juvenile *E. grevyi* (13.00). Comparison between juvenile males and juvenile females showed no significant difference ($U_{(16)} = 30.5, p > 0.05$). Likewise a comparison between foal males and foal females revealed that the difference was not significant ($U_{(16)} = 31.5, p > 0.05$).

4.9 Protozoan infections in relation to age category

The protozoan parasites included *Eimeria sp*, *Balantidium sp*, *Giardia sp*, and *Entamoeba sp*. In total, 62 protozoan parasites were recovered accounting for 12% of the total parasites. *Eimeria sp* was the dominant parasite followed by *Entamoeba sp*. Mean parasite recovery was similar in adults and the foals and higher than in the juveniles. In adult males, out of the four genera recovered two were missing and this included *Balantidium sp* and *Giardia sp*. Adult females also revealed that *Giardia sp* was the only parasite that was missing. In juvenile males all the four types of parasites were represented but in juvenile females genera *Balantidium sp* and *Giardia sp* were missing. Three genera were absent in foal males. These included *Balantidium sp*, *Giardia sp* and *Entamoeba sp*. On the other hand only *Eimeria sp* and *Giardia sp* were recovered in foal females. The highest mean parasite recovery was shown by the female foals (0.5) followed by the juvenile males (3.4) (Table 4.6).

Table 4.6: Protozoan infection of Grevy's Zebras by Age and sex categories

Age	<i>N</i>	<i>Eimeria</i> <i>sp</i>	<i>Balantidium</i> <i>sp</i>	<i>Giardia</i> <i>sp</i>	<i>Entamo</i> <i>eba sp</i>	Total	Percentage occurrence	Mean parasite recovery
Adult male	54	9	0	0	8	17	27	17/54 =0.31
Adult female	91	15	1	0	11	27	44	27/91 =0.30
Juvenile male	29	2	1	1	6	10	16	10/29 =0.34
Juvenile female	18	2	0	0	2	4	6	4/18 =0.22
Foal male	9	1	0	0	0	1	2	1/9 =0.11
Foal female	6	2	0	1	0	3	5	3/6 =0.50
Total	207	31	2	2	27	62	100	

4.10 Mean parasites recovery per category in protozoan parasites

When the parasite means of the different categories of animals were compared, the Juvenile males were found to have the highest mean (0.56) while the Juvenile females had the lowest mean (0.14). However, this difference was not statistically significant (Kruskal Wallis $H=3.27$, $P > 0.05$) (Table 4.7)

Table 4.7: Protozoan Infection

Category	N	Total parasites recovered	Mean parasites recovery x100 (%)
Adult Males	91	17	17/91 = 19
Adult Females	54	27	27/54 = 50
Juvenile Male	18	10	10/18 = 56
Juvenile Female	29	4	4/29 = 14
Foal Males	6	1	1/6 = 17

4.12 Mean Helminthes recovery per Grevyi zebra age category

There was a significant difference, ($H = 10.81$, $P < 0.05$, in the Helminthes infections) among Zebras of different age categories. Juvenile had the highest mean parasite recovery (2.54) while the adult had (2.4) and the foals had the lowest (1.83) (Table 4.9).

Table 4.9: Helminthes infection

Category	N	Total parasites recovered	Mean parasite recovery x100 (%)
Adult Males	91	107	107/91 = 118
Adult Females	54	195	195/54 = 361
Juvenile Male	18	66	66/18 = 367
Juvenile Female	29	41	41/29 = 141
Foal Males	6	14	14/6 = 233
Foal Females	9	12	12/9 = 133

Comparison between all males and all female Helminthes infestation using Mann Whitney test revealed that there was no significant difference between the sexes. However, Helminthes recovered from females 248 (57%) were higher than from males 187 (43%).

4.13 Comparison of protozoan infection between males and females

The distribution of the protozoan parasites in relation to sex revealed that there was no big difference in terms of occurrences. The females recorded a relatively higher percentage than the males but the parasite mean recovery for both was the same. *Eimeria sp* showed a higher frequency in females while in males; it was shown by *Entamoeba sp*. (Table 4.10).

Table 4.10: Number of Protozoan parasites recovered from male and female Grevy's Zebras

Protozoa species

Female	115	113	69	30	36	0	248	$248/115=2.16$
Male	92	90	46	27	23	1	187	$187/92=2.03$
Total	207	203	115	57	59	1		

CHAPTER FIVE: DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 DISCUSSION

5.1.1 Socio-demographic characteristics of the study animals

The total sampled animals were 207. They comprised of 70.0 % adults, 22.7 % juveniles and 7.3 % foals. In terms of sex, 56 % were females while 44 % were males. The lower number of foals could be due to harsh environmental conditions such as extreme heat which could claim their lives due to dehydration, predation. Adult females (mares) were more than the adult males but in juveniles, there were more males than females. The foal males on the other hand were more than the foal females. The lower number of adult males could be attributed to the fact that they established territories which were to be defended through fights and this could result to loss of lives, (Churcher, 1993). A lonely animal is prone to attack through predation by animals like lions. Generally females and their foals often gathered into herds, this gives them an advantage of group defense (Rubenstein, 2010). Their living together gives protection (Sundaresan *et al.*, 2007) and this could account for the higher numbers of females compared to males.

5.1.2 Gastrointestinal parasites infesting *E. grevyi*

The microscopic faecal examination showed that helminthes and protozoan were the dominant gastrointestinal parasites in Grevy's Zebras. The occurrence of helminthic and protozoan parasites among the Grevy's Zebras studied was 88% and 12% respectively.

5.1.2.1 Gastrointestinal helminthes infecting *E. grevyi*

Helminthic infection included *Strongylus sp.* whose occurrence was 98.1%. In this group also were *Strongyloide sp.*, *Ascaris sp.*, *Trichonema sp.* and *Trichuris sp.*, which had 55.6%, 5.3%, 5.8%, and 0.5% respectively.

These results are in agreement with the results on endoparasites in donkeys in Ethiopia which was reported to be 100%, and 98.2% in Wonchi, highlands of Wollo province and western highlands of Oromia, respectively in Ethiopia (Fikru *et al.*, 2005; Mulate, 2005). When all the samples observed under the microscope reveal the presence of a parasite of any type the recovery is said to be 100% and it decreases with the decrease in recovery of the GIT parasites. These results are similar to those obtained in studies conducted in Ethiopia and Mexico, areas with more or less similar ecological conditions as those in Samburu. The studies found the occurrence of GIT parasite infections at over 90% in horses (Fikru *et al.*, 2005; Gebreab, 1998; Valdez-Cruz *et al.*, 2006) and over 80% in donkeys (Burden *et al.*, 2010; Getachew *et al.*, 2010; du Toit *et al.*, 2008).

Gastrointestinal parasites occurrences of this type were also reported in donkeys in Gambia (Mattioli *et al.*, 1994) and Republic of South Africa (RSA) (Wells *et al.*, 1998, Matthee *et al.*, 2000). In another study of gastrointestinal parasites in horses of Sainik farm in Nigeria the occurrence of gastrointestinal parasites as 80.48% with *Strongylus sp* (48.78%), *Trichostrongylus* (31.70%), *Parascaris sp* (21.95%), *Trochonema sp* (17.07%), *Gastrodiscus* (7.31%), and *Habronema* (4.87%). As opposed to the current study, both *Gastrodiscus sp.*, and *Habronema sp* were not recovered and this could be due to variation in animal species and climatic condition of the given area. A study of parasitic infestations in equines under unorganized husbandry practices was carried out in Kashmir valley of Jammu and Kashmir state by faecal samples examination. The overall infestation was found to be as high as 93.26%. *Trichonema sp.* (96.78%) dominated other types of parasites, *Strongylus sp.* (81.19%), *Triodontophorus sp.* (41.39%), *Dictyocaulus sp.* (14.10%), *Oxyuris sp.* (9.40%), *Paranoplocephala sp.* (8.14%), *Strongyloides sp.* (6.19%), and *Parascaris sp.* (4.01%).

In another study which took over a period of one year from 1999 to 2000, faecal and blood samples of 290 race horses on 20 private horse farms were examined for parasitic infections in

Iran. The alimentary canal was the only infected organ. Faecal examination using a flotation method and saturated solution provided an indication of the *Parascaris equorum*, *Oxyuris equi*, and *Strongyle* eggs in 13.8%, 17%, and 28.3% of tested samples, respectively (Eslami, Bokai and Tabatabhai, 2000). These findings are in agreement with the current study. Studies on GIT parasites in horses indicated that stated that about 80% of Swedish horses were infected with *strongylus sp.* and *Parascaris sp.* (Roneus, 1971) while more incidences of GIT nematodes were reported (Riaz, 1984). In the present study, on Grevy's zebras that belong to the same genus with the donkeys, the results are in agreement but the occurrence of *Ascaris sp* was however lower (5.3%) than in donkeys. The variation could be due to the high heat intensity that characterizes the study area which could have reduced the number of eggs by reducing their viability and also the species difference.

Samburu region is relatively hot, this could account for decreased infection of gastrointestinal helminthes in the present study since unfavorable environmental factors such as extreme heat hinders the development and growth of most helminth species (Lima, 1998, Andrews, 1999). Most of the helminth species are susceptible to desiccation in dry climatic conditions that results from the high temperature at which even eggs fail to develop into larval stages (Banks *et al.*, 1990). The study also showed that strongyle type of nematodes was significantly higher than all the other detected helminthes. This finding is in agreement with previous studies that reported an occurrence of 100% in donkeys (Yoseph *et al.*, 2005; Ayele *et al.*, 2006; Wubishet, 2008; Getachew *et al.*, 2009) in different parts of Ethiopia which has a similar ecological condition as in Samburu region. This is most probably attributed to the lack of intervention measures with anthelmintics in the region due to its remoteness. This observation of high parasitic occurrence also agrees with several previous reports from different parts of the world on donkeys (Eysker and Pandey, 1989; Lyons *et al.*, 2000; Meana *et al.*, 2005). In the current study, *ascaris sp* had the occurrence of 5.3%. This parasite was also recovered in

horses in different studies but with variation in occurrence. The findings, however, reflected a lower occurrence compared to the 53% *Parascaris sp* recorded in previous reports (Ayele *et al.*, 2006; Zerihun, 2008) that reported 43 and 42.8% in Dugda Bora district and highlands of Wollo provinces respectively in Ethiopia. Studies involving horses endoparasites reported occurrence of *ascaris sp* at 17.1% in the Ethiopian highlands (Fikru *et al.*, 2005) and another finding (Getachew, 2008) reported an occurrence of 16.2% (Getachew, 2008). Reported occurrence of ascaris in donkeys within four sites in Ethiopia were higher and they stood at 43.5% (Gebreab, 1998), 50% (Ayele, 2006), 51.1% (Getachew, 2008) and 51% (Getachew *et al.*, 2010). The current study however has shown that all the age categories have been affected and this could be related to immunity differences in species. Studies on horses in Riyadh region of Saudi Arabia also found the presence of parasites like those detected in Samburu region. The most prevalent parasites were *Strongyloides sp* (64.4%) and *Ascaris sp* (28.8%).

The current study has shown agreement with these findings which has indicated an occurrence of 55.6% *strongyloides sp* and 5.3% *ascaris sp*. In another study, faecal samples were collected from fifty-four stallions randomly within Sokoto metropolis. After analysis, helminth ova identified were those of *Ascaris sp*, *Strongylus sp*, *Strongyloide sp*, *Panaplocephala sp*, *Dictyocaulus sp*, and *Gastrodiscus sp*. 84.4% of the samples examined were positive, out of which *Strongylus sp*. had the highest (75.5%) percentage occurrence. This study of horses in Sokoto metropolis was aimed at having an insight into the most prevalent gastrointestinal helminthes and to give recommendations towards improving the health and management of horses (Alayande, Onakpa and Lawal, 2003). The recovered parasites were similar to the ones in the current study in the Samburu region, the absence of some could be due to differences in feeding behaviour in relation to parasite ingestion. The current study indicated the occurrence of *Trichuris sp* as 0.5%. This is however a lower occurrence compared to studies on Gastrointestinal Parasites of Sheep and Goats in and

around Bedelle, South-Western Ethiopia where the occurrence of *Trichuris sp.* was 47.8% and 45% in sheep and goats respectively. The recovery of these parasites is in harmony with the reported work on sheep and goats endoparasites which gave 36.04% and 28.57% respectively in Ogaden region of Ethiopia (Esayas, 1988; Hailelul, 2002). The variation in these results could be due to differences in species, immunity levels and climatic conditions among others. In the current study, the sheep, goats and the Grevy zebras share the same habitat and hence there is a higher chance of cross transmission of these parasites. The current occurrence of gastrointestinal nematodes results also agrees with reports of previous donkey studies conducted in Ethiopia. It reported 56.6% *strongyles sp.*, 8.2% *strongyloides sp.* and 5% *trichuris sp.* (Tigist, 2008). In another study of donkeys in the same area, 66.6% *strongyles sp.* type and 3.3% *Trichuris* species were observed (Temesgen, 2008); A study on GIT infection in equids in Ethiopia revealed 64% *strongyles sp.* type, 7.4% *strongyloides sp.* and 3.7% *Trichuris sp.* (Diriba and Birhanu, 2013) this work was done in Asella, south eastern part of Ethiopia.

This difference in occurrence could be due to the fact that, occurrence varies greatly from region to region, corresponding to ecological differences, species variations and climatic diversity as well as the existing host ranges (Njau *et al.*, 1990). Trematodes and cestodes were not detected in this study. This could be due to the fact that these parasites require an intermediate host for their transmission which could be absent in this environment (Cordon *et al.*, 2008). The gastrointestinal parasites species identified in this study have previously been recorded in captive animals in various zoos and zoological garden by other authors. Hot environmental conditions are conducive for the development of the pre-parasites stages (Singla, 1995). The current study area being hot could be probably more conducive to some types of parasites like the strongyles and this could account for their high frequencies.

5.1.2.2 Gastrointestinal protozoans infecting *E. grevyi*

The protozoan parasites on the other hand which included *Eimeria sp*, *Entamoeba sp*, *Balantidia sp* and *Giardia sp*, had occurrences of 15 %, 13%, 1.0 % and 1.0% respectively.

A study of parasitic infestations in equines in Kashmir revealed that the faecal samples habuored *Amphistome sp.* (0.91%) and *Eimeria sp.* (0.34%) were also recorded (Pandit *et al.*, 2008; Souza *et al.*, 2009).

A horse study on GIT parasites in Brazil found the prevalence rate of *E. leuckarti* smaller than 1%. The Wide spread *E. leuckarti* infection in several countries has been ranging from 2% to 80% (Germany), 64.9% to 80% (Greece), 3.1 %, (Poland) and 6.7 % (Turkey) among other regions (Studzinska *et al.*, 2008). These results agree with the current study that reported an occurrence of 15% which is within the given ranges but the disparity could be attributed to variation in the ecology of the region, climatic factors and species difference. It is believed that the infection of coccidian concerns mainly young horses and especially foals. It has been reported that *E. leuckarti* is more prevalent among young foals and only occasionally detected in adult animals (Souza *et al.*, 2009). These findings are similar to the ones in the current study and the minor differences could be linked to geographical variations.

5.1.3 Parasite load distribution per age category

The current study revealed parasite load decrease from adults to foals which ranged from 1200 to 1600 where the adult and the juveniles registered severe degree of infection (92.8%) while the foals (7.2%) had moderate infection. There was no case of light infection. The foals had 1200, juveniles, 1300 while the adult had 1600 EPG count. This study also indicated a significant difference in infestation levels among age categories with the adult having a higher infestation compared to the juveniles and the foals. Such similar findings were reported in cattle (share habitat and pasture with the study animals) whose EPG count from semi-arid parts of Kenya was reported to be of higher intensity in older animals than young ones

(Collobert *et al.*, 2000; Magona *et al.*, 2005). They also noted that the occurrence of small *Strongyloides sp* significantly increased with age. A study involving EPG count done in Kashmir region on donkeys revealed a range between 200 and 800 (Pandit, 2008). This is however lower compared to the current study. Potential variation arising from species differences and differences with respect to topography, climate, animal working conditions as in the case of donkeys and seasonality make direct comparisons difficult. The occurrence of various endoparasites and the high mean egg per gramme count of *strongyles sp* in the animals may suggest lack of immunity to this parasite (Uslu and Guclu, 2007). Work on donkey endoparasites in Ethiopia revealed that the greatest proportion of young donkeys were with severe degree of infection (51.8%) followed by moderate degree (29.6%) whereas the majority of adult and old donkeys had mild degree of infection (28.57%). Similar observations were seen in previous research (Matthee *et al.*, 2002; Getachew *et al.*, 2009). It was suggested that this could be due to the fact that the young donkeys have less immunity against helminthes than both adult and old donkeys since the highest mean strongyle egg count was observed in young age group.

The current study, however, revealed highest parasite load in adult grevy zebras. This variation could be attributed to the fact that wild animals have relatively higher degree of immunity than domesticated animals, hence can harbor bigger loads without showing signs of infection. The species difference, the climatic variation, the feeding habits and the free intermingling of these animals in the wilderness gives them a higher chance to share the parasites. The influence of climatic conditions on the dynamics of egg expulsion has also been reported (Klei and Chapman, 1999; Kuzmina, 2006). They reported that the naturally acquired immunity against small strongyles slowly develops with age, but remains incomplete. Domesticated animals which include the donkeys may get some forms of protection depending on the keeper against parasitic infection like the use of anthelmintics compared to

those in the wilderness that cannot get such services. This could provide the explanation for these findings of high parasite infestation levels in adults in the current study. A study on faecal egg count (FEC) in horses over a period of two years in Ethiopia confirmed that counts were higher in the long rainy season as compared to the dry season (Getachew *et al.* 2008).

Previous reports for the severity of infection in equids in Ethiopia vary (Gebreab, 1998), he recorded 51% of 157 horses as having severe infection. A more recent study (Fikru *et al.*, 2005) reported only 29% of horses in Ethiopia to have a heavy infection. In another study in Ethiopian donkeys, it was reported that over 55% of them had more than 1000 eggs per gram (Getachew *et al.*, 2010). This is a similar finding with the current study that also showed over 50% severity of infection to the study animals.

Studies on donkey endoparasites around Suluta region of Ethiopia showed that the greatest proportion of young donkeys were with severe degree of infection (51.8%) followed by moderate (29.6%), whereas the adult (28.57%) had mild or light infection (Mathee *et al.*, 2002). This is in contrast with the current findings of the study whose EPG count was higher in adult compared to the other categories. The differences in intensity of infection could be due to variations in parasite biology relating to climatic conditions, pasture infection intensity relating to grazing practices or differences in use of anthelmintics as in the cases of domesticated equines like the donkeys. Climatic and environmental differences between countries and differences in access to drugs and the extent of interventions by equine charities may partly explain the variation in these estimates.

The EPG counts also are affected by season with significant rise in wet season. Likewise, seasonal fluctuations in nematode faecal egg counts which followed seasonal rainfall pattern were reported from different studies in Ethiopia (Fikru *et al.*, 2006). The current study was carried out during dry period and prolonged drought resulting in wilting of

vegetation. This forced the animals to graze closer to soil that is potentially infected with parasites (Dragon and Rennie, 1995). This further suggested that, although other conditions could possibly account for increased susceptibility to parasitic infection, grazing behavior has played a more important role in exposing the study animals to heavy infections. Studies have shown that the risk of gastrointestinal parasite infections increase with grazing close to the ground compared with browsing shrubs and trees (Apio *et al.*, 2006). In the wild also, parasites rarely build up to the point of seriously damaging their hosts' health. Domestic equines on the other hand find parasites a potentially devastating threat to their health, largely because they are kept in confined spaces and are not exposed to challenges of the wilderness to harden enough. This could also account for the high EPG count in adults of the current study animals. In some cases Egg counts are of limited value in making judgments about the clinical condition of individual animals because many factors affect parasite egg production, including parasite species, individual host species, host immunity and stage of infection. Also, counts performed on combined samples from a number of animals may not accurately reflect parasites within that herd. Individual animals produce higher egg counts (high shedders) than others (low shedders) (Krecek *et al.*, 1994).

5. I.4 Infestation levels among the various age and sex categories of *E. grevyi*.

Some parasitic infections have inclination to age groups. Regarding *ascaris sp*, infection in horses that are in similar generic category with the grevy zebras have shown that parasitic infestation is a problem only for young horses. It was however reported that horses develop excellent acquired immunity to ascaris infection resulting in infections being limited to suckling, weanlings and yearlings and being observed only occasionally in horses older than two years (Reinemeyer and Nielsen, 2009). This could be due to the fact that domesticated animals like the horses at some point get treated by the owners as opposed to the wild equines whose infestation levels rises day by day without intervention. This may account for the

observed variation in parasitic infection within the different age categories of the study population. In Ethiopia, high occurrences were reported in younger animals (Gebreab, 1998; Fikru *et al.*, 2005) whereas in another study, no association was established between ascaris infection and age in neither the horses nor the donkeys (Getachew *et al.*, 2008; Getachew *et al.*, 2010) which belong to the same genus with the Grevy zebras.

The current study shows that the adult animals were seen to harbor more of these parasites than the young ones, this could be due to the fact that the adult spend most of their day feeding on grass that in some cases is so short due to drought forcing the animal's mouth to be in contact with the ground. This raises their chances of these animals ingesting the parasites eggs resulting to high infestation levels as observed in the current study animals.

5.2 CONCLUSIONS

- (i) Both helminthic and protozoan type of gastrointestinal parasites were prevalent in the Grevy's zebras of the Samburu landscape. The protozoan type recovered included, the *Eimeria leuckarti*, *Entamoeba equi*, *Balantidium coli* and *Giardia intestinalis* while the helminthic type included *Strongylus vulgaris*, *Strongyloide westeri*, *Trichonema sp*, *Parascaris equorum* and *Trichuris trichiura*.
- (ii) The mean parasite loads for various age categories revealed that the adult had higher parasitic loads than the juveniles and the foals. Both the adult and the juveniles had severe infection while the foal had moderate infection. There was no category with light infection.
- (iii) It was also found that the infestation level of parasites between males and females in *E. grevyi* zebras was the same.

5.3 RECOMMENDATIONS

5.3.1 Conservation and management

- i. A comprehensive parasite control program should include environmental sanitation, and regular antihelminthic administration.
- ii. Basic knowledge of animal health could be introduced to the pastoralists since they help in the conservation activities to detect ill health and report to the necessary authorities.
- iii. For prevention of spread of gastro-intestinal parasites, the contamination of pasture land should be prevented by treating the hosts with antihelminthics. . In this regard, the Samburu pastoralists should be encouraged to use antihelminthics to their animals since they share similar habitat with the Grevy zebras and this will reduce cases of cross transmission of the parasites.
- iv. Government or non-profitable development agencies should include Grevy zebras in their priority lists of research and develop sustainable integrated diseases prevention and control programs that are practical for developing communities.
- v. Based on the given data, a detailed survey is recommended to establish other parasitic problems and diseases affecting the animals.
- vi. The role and importance of zebras for parasitic transmission and their significance in the continuity of some zoonotic diseases needs to be investigated.

5.3.2 Suggestions for further study

Following the discussion given above, parasitic infection is a broad field in equines and one would always leave gaps in some areas. It is out of this context that more knowledge would

always be necessary and worth visiting. A reason why the following were suggested to boost one's understanding;

- What are some of the GIT parasites infecting donkeys within the Samburu region? Donkeys are related to the study animals since they have the same genus. They are the ones that have been used to support the findings and hence it is worthy to consider them separately in this region.
- Is there cross transmission of parasites from domestic equids and the Grevy's zebra within the Samburu landscape? Grevy zebras share habitat with other domesticated animals like goats, sheep and camels. However it has not been established whether there is cross transmission of the GIT parasites.
- Do the gastrointestinal parasites affect the Grevy's zebra growth, reproduction and survival within the Samburu landscape? The study population has shown that there are less number of foals compared to the juveniles and adults but it is not clear whether their development and survival rates are related to parasitic infections.
- Does seasonal variation affect parasitic infestation levels in Grevy's zebras in the study area? This study was conducted mainly during dry season. The variation in seasons within the Samburu region could affect the infestation levels of the parasites.
- Does pasture management reduce GIT parasite infestation levels in Grevy's Zebra? Samburu region is located in a remote area that is not easily accessible. The findings of this study could be affected by pasture management.

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APPENDIX I : Materials**Materials**

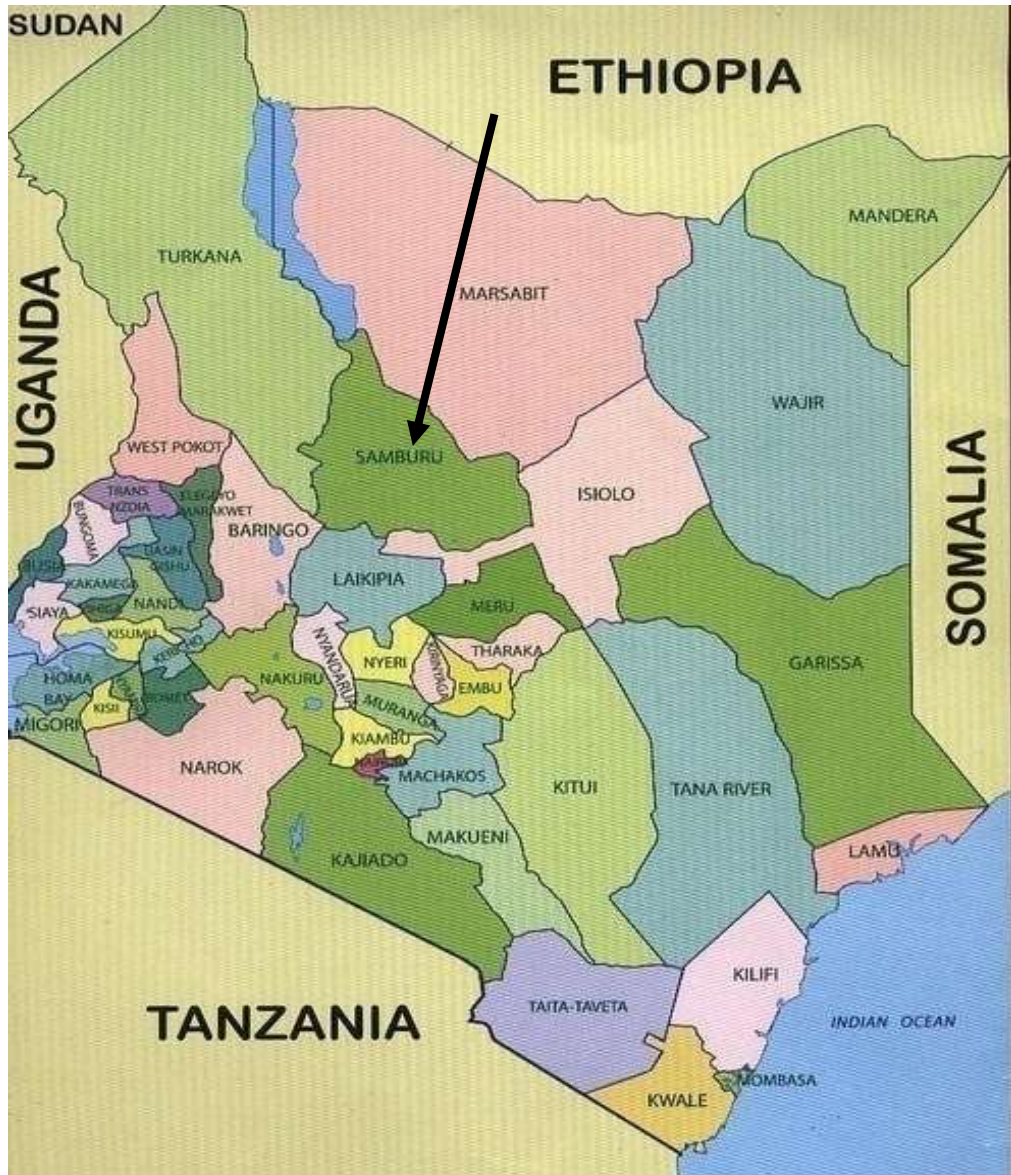
- i. Polypots
- ii. 15ml centrifuge falcon tubes
- iii. Beakers
- iv. Applicator sticks
- v. Cheese cloth/gauze or sieve
- vi. Screw-capped bottles
- vii. 10% formalin
- viii. Microscope (Omega light microscope)
- ix. Slides and cover slips
- x. McMaster chamber
- xi. Lugol's Iodine solution
- xii. Gloves
- xiii. Paper face masks
- xiv. Disinfectant (10% jik or 5% savlon)
- xv. Normal saline/physiological saline (0.85% sodium chloride)
- xvi. Centrifuge machine 80-2 China
- xvii. Cool boxes
- xviii. Ice packs
- xix. Weighing balance- Ohaus –CS 200-China
- xx. Distilled Water
- xxi. Glass rods
- xxii. Diethyl-ether GPR

APPENDIX II : Dung Collection Data Sheet

Dung Collection Data Sheet

Sample no.	Date	Area	GPS N	GPS E	Age			Repro. status	Body Cond.	Comments
					Adult	Juv.	Foal			
					1.Female			1. Lactating	1.Poor	
					2.Male			2.Non-lactating	2. Moderate	
								3.Pregnant	3. Ideal	
								4. Territorial	4. Fat	

APPENDIX III : Map of Kenya showing the study area



APPENDIX IV : PICTURES DURING SAMPLE COLLECTION AND ANALYSIS



Close follow up of the Grevy zebras in a Landrover to get the samples

Laboratory Setting for Sample Analysis



APPENDIX V : STATISTICAL TESTS

Kruskal-Wallis Test: number of parasites versus age

Foals	juveniles	adults
10	35	167
4	8	43
2	2	12
13	59	91
2	9	25
0	8	8
H	6.468	
Hc	6.522	
P	0.038	

Mann-Whitney Test: number of parasites versus sex

Female	male
167	91
43	25
12	8
35	59
8	9
2	8
10	11
4	0
2	0

U = 40 df = 16

U 0.05 9, 9 = 64

Kruskal-Wallis test on Helminthes infection with age categories.

Foal	Juveniles	Adults
12	46	78
2	20	28
6	18	90
4	17	78
2	6	28

H = 10.81
 Hc = 10.89
 P = 0.004312

Kruskal-Wallis test on protozoa infections with age categories

Foal	Juveniles	Adults
1	2	9
0	1	0
0	1	0
0	6	8
2	2	15
0	0	1
1	0	0
0	2	11

H 3.279

Hc 3.582

p (Same) 0.1668

Mann-Whitney test on Helminthes infection with sex categories

Female	Male
143	78
52	28
18	29
17	31
6	7
9	12
3	2
0	0

$U = 30.5$ $df = 14$

$U_{0.05, 8, 8} = 51$

Mann-Whitney test on Protozoa infection with sex categories

Female	Male
16	9
11	8
2	3
2	7
2	1
1	0

$U = 16.5$ $df = 10$

$U_{0.05, 6, 6} = 31$

Mann-Whitney test on parasite infection on adult males and females

Adult males Adult Female

54 89

24 54

13 24

15 28

1 0

9 15

8 11

0 1

0 0

U = 32 df = 16

 $U_{0.05, 9, 9} = 64$ **Mann-Whitney test on parasite infection on juvenile males and females**

Juvenile male Juvenile female

28 18

18 12

13 5

7 6

0 0

2 2

6 2

1 0

1 0

U = 30.5 df = 16

 $U_{0.05, 9, 9} = 64$

Mann-Whitney test on parasite infection on foal males and females

foal male foal female

8

6

4

3

1

1

1

2

0

0

1

2

0

0

0

1

0

1

 $U = 31.5 \quad df = 16$ $U_{0.05, 9, 9} = 64$

APPENDIX V1 : PARASITE GROUPS

Protozoan Parasites

Parasite (scientific name)	Type
<i>Entamoeba histolytica</i>	Amoeba
<i>Giardia duodenalis</i>	Flagellate
<i>Trypanosoma brucei</i>	Flagellate
<i>Trypanosoma cruzi</i>	Flagellate
<i>Leishmania spp.</i>	Flagellate
<i>Eimeria spp.</i>	Apicomplexan
<i>Isospora spp.</i>	Apicomplexan
<i>Cryptosporidium spp.</i>	Apicomplexan
<i>Toxoplasma gondii</i>	Apicomplexan
<i>Plasmodium spp.</i>	Apicomplexan
<i>Babesia spp.</i>	Apicomplexan
<i>Balantidium coli</i>	Ciliate
<i>Ichthyophthirius multifiliis</i>	Ciliate
<i>Thelohania spp.</i>	Microsporan

Helminthes

Parasites (scientific name)	Group	Common name
<i>Ascaris lumbricoides</i>	Nematode	roundworm
<i>Trichuris trichiura</i>	Nematode	whipworm
<i>Enterobius vermicularis</i>	Nematode	Pinworm
<i>Ancylostoma duodenale</i>	Nematode	Old World hookworm
<i>Necator americanus</i>	Nematode	New World hookworm
<i>Strongyloides stercoralis</i>	Nematode	threadworm
<i>Dracunculus medinensis</i>	Nematode	guinea worm
<i>Onchocerca volvulus</i>	Nematode	filarial worm
<i>Echinococcus granulosus</i>	Cestode	dog tapeworm
<i>Taenia saginata</i>	Cestode	beef tapeworm
<i>Taenia solium</i>	Cestode	pork tapeworm
<i>Fasciola hepatica</i>	trematode	liver fluke
<i>Schistosoma</i> spp.	trematode	blood fluke