UNIVERSITY OF NAIROBI

PREVALENCE AND DIVERSITY OF HAEMOPARASITES AND GASTROINTESTINAL PARASITES IN DONKEYS CO-GRAZING WITH ZEBRAS AT AMBOSELI NATIONAL PARK AND ADJACENT AREAS

By

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Declaration

I declare that this thesis is my original work and has not been submitted elsewhere for examination or the award of a degree. Where other people's work has been used, this has been properly acknowledged and referenced in accordance with the requirements of the University of Nairobi.

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ABSTRACT

Donkeys (*Equus asinus*) play an important role as a mode of transport in many communities, however their role is constrained by illness due to parasitic infections which can also affect other related wild equid species such as zebra often found co-grazing together with donkeys. Lack of a boundary fence at the Amboseli National Park (ANP) allows zebra migration out of the park and interaction with donkeys and other domestic animals within the adjacent community, with the possibility of transmitting infections between them. This study determined the prevalence and described the diversity of parasites occurring in sympatric zebras and donkeys at the ANP and adjacent areas. Fifteen blood samples were collected opportunistically from plains zebras (*Equus quagga*) during Kenya Wildlife Service scheduled management activities. Seventy-Nine donkeys were sampled from communities adjacent to ANP. Blood samples were screened for *Theileria*/*Babesia* and *Ehrlichia*/*Anaplsma* species by PCR amplifications and sequencing of the 18s and 16s rRNA gene respectively. Faecal samples were collected from both zebras and donkeys and analyzed for gastrointestinal parasites (GIP) using the sugar flotation technique and the modified sedimentation technique. Ticks were collected from 3 zebras and 38 donkeys and identified using morphological characteristics under a stereomicroscope and separated into species and developmental stages then screened for presence of tick-borne pathogens. *Ehrlichia*/*Anaplasma* was detected in 5 % of donkeys, but not in zebras. A partial 16S rRNA sequence similar to that of *Anaplasma cf. platys* was identified only in 4 % of the donkeys. *Theileria*/*Babesia* was detected from 92% donkeys, and 93%in zebras with a prevalence of *T. equi* at 87%, and 84 % respectively. The overall prevalence of gastrointestinal parasites was close to 100% in both zebras and donkeys. Gastrointestinal parasites identified from zebras included *Anoplocephala* sp., *Parascaris* sp., strongyle, *Trichostrongylus* sp., *Dictyocaulus* sp., trematode, *Oxyuris equi*, and *Habronema* sp. while *Anoplocephala* sp., *Parascaris* sp., strongyle, *Trichostrongylus* sp., *Dictyocaulus* sp., *Oxyuris equi*, and *Strongyloides* sp. were detected in donkeys. Strongyle was the most found gastrointestinal parasite in both zebra and donkey, while *Habronema* sp. and *Dictyocauls* sp. was only found in zebras and potentially zoonotic *Strongyloides* sp. was only found in donkeys. *Rhipicephalus pulchellus*, *R. praetextatus*, *R. evertsi*, *Hyalomma albiparmatum*, *H. impeltatum*, *H. rufipes*, and *Amblyomma gemma* ticks were collected from zebras while *R. pulchellus* and *R. praetextatus* were found in donkeys. Further studies to characterize the *Strongyloides* sp. and the potential reservoir role of donkeys should thus be considered within the preventive and control measures of zoonotic parasitic infections.

DEDICATION

This thesis is dedicated to my mother, Setsuko Kono and my father Tomoyuki for their sacrifices towards my education.

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I gratefully acknowledge the management of The Amboseli National Park, members of the veterinary laboratory at Kenya Wildlife Service, Sheldrick Wildlife Trust, the donkey owners around The Amboseli National Park for the kind assistance accorded.

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LIST OF ABBREVIATIONS

ANP – The Amboseli National Park

BHC – Benzene hexachloride

CO1 – Cytochrome Oxidase Subunit 1

CO3 – Cytochrome Oxidase Subunit 3

DDT – Dichlorodiphenyltrichloroethane

ELIZA – Enzyme-linked Immunosorbent Assay

GIP – Gastrointestinal Parasites

IFA – Indirect Fluorescent Antibody

ITS1 – Internal Transaction Spencer 1 Gene

ITS2 – Internal Transaction Spencer 2 Gene

L1 – Larva1

L3 – Larva 3

L4 – Larva 4

L5 – Larva5

LAMP – Loop-mediated Isothermal Amplification

PCR – Polymerase Chain Reaction

Plains Zebra – Zebra

RLB – Reverse Line Blot

CHAPTER ONE

INTRODUCTION

1.1. Background Information

Three-quarters of around 1.2 million donkeys (*Equus asinus*) found in Kenya are used for transport and farming. Donkeys play a key-role in Kenya agriculture with one-third of the people who work with donkeys being poor. Recently, there is a high demand for donkey meat in Kenya making these equids more valuable. However, they equally suffer diseases that heavily affect the poor and principally affect agricultural output. The most common diseases affecting the donkeys are tetanus, tick-borne pathogens, fever, African horse sickness, helminth and rabies (Saul *et al*., 1997).

Equine piroplasmosis is tick-borne haemoparasites that affects domestic and wild equid species. Equine piroplasmosis cause a significant economic impact on the donkey population due to mortality. The world organization for animal health (OIE) being an organization for improving animal health around the world, has enlisted equine piroplasmosis as a disease of importance. Equine piroplasmosis is an economic importance disease for equid species. However, there is little or no information on Equine piroplasmosis among livestock and wild animals in Kenya since 2006, according to the World Animal Health Information System report. Notwithstanding,

Oduori *et al*., (2015) reported *Theileria equi* in 81.2% of donkeys in Mwingi district. Hawkins *et al*., (2015) demonstrated the prevalence of *T. equi* in 72% of donkeys and 100% of Grevy's zebras (*Equus grevyi*) in the Wamba area. These studies, therefore, demonstrated that equine piroplasmosis could be a potential threat to the donkey and plains zebra (*Equus quagga*) (zebra) population in Kenya.

Other equine vector-borne diseases such as *Anaplasma phagocythophilum*, *Babesia caballi* and *Borrelia* spp. have been detected from equids (Motloang *et al*., 2008; Laus *et al*., 2013; Veronesi *et al*., 2014). In Egypt, about 30% donkeys infected *B. caballi* (Mahmoud *et al*., 2016).In South Africa, *B. caballi* were also detected from a few zebras (Zweygarth *et al*., 2002). In Italy, *A. phagocythophilum* has been detected from donkey blood (Torina *et al*., 2008). Lewa *et al*., (1999) and Ahmed *et al*., (2008) reported other haemoparasites that appeared to pose a health risk to donkeys. This, therefore, demonstrate that, not only do Equine piroplasmosis but other haemoparasites could affect the health of donkeys and zebras.

Most haemoparasites are transmitted by arthropods especially ticks. Norval *et al*., (1992) reported equine piroplasmosis transmitted by several tick species such as *Amblyomma, Rhipicephalus, Boophilus,* and *Hyalomma* species. Lightfoot and Norval (1981) detected and reported *R. evertsi*, collected from zebra in Zimbabwe. There is a

likelihood that serious tick problems prevail in several areas where wild animals live. Tick species differ among different areas because of climate and animal host species. Some ticks are host specific, but others are not. This, therefore, allows ticks to transmit parasitic pathogens from other animals to zebra and/or donkey. It is therefore assumed that areas with sympatric zebra and donkey distributions could increase donkey infection with equine piroplasmosis than areas where there is no co-habitation.

Gastrointestinal parasites shared between equid species can lead to a reduction in work output, discomfort and pain. All common helminth parasites could infect and cause diseases in the affected donkeys. A study conducted in Samburu (Knafo, 2008) demonstrated that zebra shared similar intestinal helminth parasites with donkeys. Lichtenfels (1975) reported that equids had more than 75 helminth species. The most common helminth species of equids identified were, ascarid, lung worm, tapeworms (Cestoidea, Fasciolidae, Anoplocephalidae) and pin worm (*Strolgiloides* sp.). Depending on the species and intensity of infection, these parasites could cause damage to the intestine (Pereira and Vianna, 2006; Ayele and Dinka, 2010; Mekibib *et al*., 2010). Donkeys sharing common habitats with zebras are likely to be infected with similar gastrointestinal parasites.

Parasite infections can cause substantial morbidity and mortality in donkeys (Chitra *et al*., 2011), with differences in the prevalence of diseases due to ecological and climate locations (Ibrahim *et al*., 2011).

Antibodies to *T. equi* were detected in human who is a veterinary in Italy (Gabrielli *et al*., 2014), suggesting a potential risk of zoonotic transmission due to close interaction between donkeys, zebras and humans at the human-wildlife and livestock interface.

The Amboseli National Park is not a fenced and wild and domestic animals migrate into and out of the park, while sharing common grazing grounds. It will be possible to be transmitted diseases between these wild and domestic animals.

1.2. Problem Statement

Ticks and haemoparasites like Equine piroplasmosis cause major economic losses to domestic equines such as donkeys. Zebras are a known reservoir for *Theileria* species affecting domestic equines in Africa (Young *et al*., 1973). The gastrointestinal parasites cause diseases and reduce working output in domestic donkeys. Therefore, infections that include haemoparasites and gastrointestinal parasites are a threat to zebras and donkeys in Kenya.

1.3. Justification

There is a paucity of information on ticks, haemoparasites and gastrointestinal parasites of equid species in Amboseli, Kenya. Investigation on common species and prevalence rate of equine haemoparasites and gastrointestinal parasite of zebras and donkeys will improve disease understanding thereby improving treatment and control. Information on parasitic infections in donkeys and zebra will lead to increased veterinary knowledge, while also informing treatment and control options.

1.4. Research Questions

1. What tick species infest zebra and donkey within the Amboseli ecosystem?
2. What tick transmitted parasites are found in zebra and donkey?
3. What gastrointestinal parasites are found in zebra and donkey?

1.5. Study Objectives

1.5.1. General Objective

To determine the diversity and prevalence of ticks, haemoparasites and gastrointestinal parasites in donkey co-grazing with zebra at the Amboseli National Park

1.5.2. Specific Objectives

1. To determine the species of ticks infesting zebra and donkey at the Amboseli National Park and its adjacent areas
2. To establish the haemoparasites identified from zebra and donkey blood samples
3. To establish the gastrointestinal parasites identified from zebra and donkey faecal samples

CHAPTER TWO

LITERATURE REVIEW

2.1. Economic Importance of Donkeys and Zebras in Kenya

According to the last population census of domestic animals in Kenya, the donkey population is estimated at around 1.2 million donkeys (Kenya Agriculture and Livestock Research Organization, 2019). Many communities use donkeys for transportation, treatments, fetching water, and various other reasons (Nengomasha *et al*., 2000). For example, Maasai women in Kenya give donkey milk to children with pneumonia or severe cough for disease prevention and treatment. The slaughter of donkeys for meat and skin in Kenya has recently increased owing to the high market demand for these products from China. In addition to being a tourist attraction, zebras also play an important role in the ecosystem by fertilising the ground, thus enabling the production of soft new leaves and grass growth.

2.2. Parasites of Equids

Donkeys and zebras belong to the family Equidae, which is a taxonomic family of horses and other related animals. The most common parasites in equids are *Trypanosoma* spp., *Giardia* spp., *Eimeria* spp., *Isospora* spp., *Cryptosporidium* sp., *Toxoplasma* spp., *Neospora* spp., *Theileria* spp., *Babesia* spp.,, *Strongylus* spp., all of

which cause infections (Sazmand *et al*., 2020). Gastrointestinal parasites can cause adverse health effects in donkeys. Over working causes parasitic infections to become severe; however, these parasitic infections can still be severe even when the donkeys are allowed to rest and graze freely on pasture. Therefore, the potential to become infected with gastrointestinal parasites is likely to be higher than that of other diseases. Moreover, VanderWaal *et al*., (2014) reported that zebra had the highest frequency of infection with helminths, and there were no helminth-free zebras observed in their study.

Donkeys are associated with several zoonotic diseases including tick-borne diseases (Morsy *et al*., 2014; Helmy *et al*., 2017). Donkeys are ata high risk of tick bites and infection from tick-borne pathogens because they are frequently kept outdoors. If donkeys share habitats with zebras, it is possible to share not only gastrointestinal parasites but also equine piroplasmosis. Equine piroplasmosis can cause economic loss in the equine industry. Beugnet and Marié (2009) reported that equine vector-borne diseases are often zoonotic, and equids are potential hosts of some pathogens that can lead to human infections.

2.3. Haemoparasites

Equine piroplasmosis is a disease caused by *Babesia caballi* and *Theileria equi*. Ticks are the definitive vectors of equine haemoparasites, which undergo sexual

development and sporozoite replication in the tick salivary glands and are transmitted to the host when the tick bites. The symptoms of *T. equi* and *B. caballi* infection are fever, dyspnoea, and hyperaemia of the mucosal membranes.

2.3.1. Life Cycle of *Theileria equi*

The life cycle of *T. equi* is as follows: erythrocytes pass through the tick midgut after sucking blood from the infection host, allowing the parasites to develop to the sexually active stage. The gamonts fuse and form zygotes, which invade the midgut epithelial cells. After invasion, the parasites develop into kinetes. Kinetes then enter the tick salivary glands and transform into sporozoites. Sporozoites are the stage of infection of the animal host. When the infected tick feeds on the host, the equid becomes infected with *T. equi* (Fig 1).

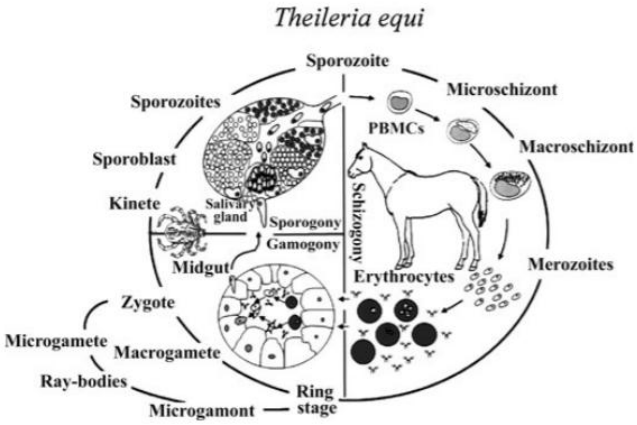


Figure 1: The representative of the image of the life cycle of *Theileria equi* (Wise *et al*., 2013)

2.3.2. Life Cycle of *Babesia caballi*

The life cycle of *B. caballi* is similar to that of *T. equi*. The difference in *B. caballi* is that the kinetes make their way into the haemolymph and infect multiple internal organs, including the ovaries, instead of invading the salivary glands. Infection of the eggs in the ovary of the tick causes vertical transmission. In tick embryos, kinetes enter the salivary glands, and the parasites grow into sporozoites which are then at the stage of infection (Fig 2).

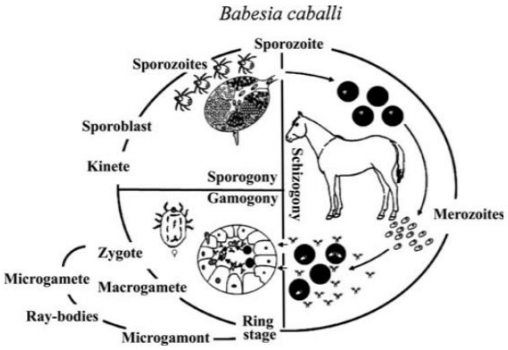


Figure 2: The representative of the image of the life cycle of *Babesia caballi* (Wise *et al*., 2013)

2.3.3. Other Tick-Borne Pathogens in Equid Species

There are three other tick-borne haemoparasites within the genera *Ehrlichia*, *Anaplasma*, and *Neorickettsia*. The genus *Ehrlichia* belongs to order *Rickettsiales*. Some species of *Ehrlichia* have been found within equid species: *E. ruminantium*, *E. chaffeensis*, *E. canis,* and *E. ewingii* (O'Nion *et al*., 2015). In addition, some *Ehrlichia* spp. have been classified into the genus *Anaplasma* or *Neorickettsia*. *N. risticii*,

formerly *E. risticii*, is characterised by fever, depression, diarrhoea, and leukopenia. Similarly, *E. equi* together with *E. phagocytophilum*, which causes disease in ruminants, has become the single species *Anaplasma phagocytophilum*. When a tick consumes a blood meal, *A. phagocytophilum* is transmitted from the infected host. If the parasite is transferred at the larva or nymph stage, continues to metamorphosis inside the tick. The next stage is the transmission of the parasites to uninfected host animals via blood feeding. The dead-end host of *A. phagocytophilum* is humans. The symptoms of *A. phagocytophilum* include limb oedema, anorexia, depression, ataxia, fever, mild anaemia, thrombocytopenia, and leukopenia, which have been demonstrated in equine species.

2.3.4. Tick-Borne Haemoparasites Reported in Zebras and Donkeys from Previous Studies

Young *et al*. (1973) discovered *T. equi* in the Cape Mountain zebra (*Equus zebra zebra*), and it has also been detected in Grevy's zebra in Kenya (Hawkins *et al*., 2015). *B. caballi* and *T. equi* have also been detected in Burchell's zebra (*Equus quagga burchellii*) and Cape Mountain zebras in South Africa (Lampen *et al*., 2009; Bhoora *et al*., 2010). Tirosh-Levy *et al*. (2019) reported that *T. equi* was detected in 32% of donkeys, 89% of Asiatic wild donkeys (*Equus hemionus*), 89% of African wild donkeys

(*Equus africanus*), and 62% of the zebras in Israel.

In Ethiopia, several studies have detected *B. caballi* and *T. equi* in donkeys using blood smears and serological tests (Mekibib *et al*., 2010). Nakayima *et al*. (2017) reported 25 donkeys infected with *T. equi* in Uganda; however, all were negative for *B. caballi*. In Kenya, Oduori *et al*. (2015) found that 81.2% of donkeys were infected with *T. equi*, but *B. caballi* could not be detected in the donkeys by means of enzyme-linked immunosorbent assay (ELISA).

2.3.5. Diagnosis of Tick-Borne Pathogens

Blood and organ smears are common diagnostic tests for tick-borne pathogens in a host, using Giemsa, Wright's, or Diff-Quik® stains. However, this morphological method makes the detection of tick-borne pathogens difficult (Rucksaken *et al*., 2019). Polymerase chain reaction (PCR) is sensitive and specific than morphological methods. Reverse line blot, multiplex PCR tests, and loop-mediated isothermal amplification can detect several pathogens in addition to PCR.

Additionally, serological tests are useful in clinical cases. In serological tests, indirect fluorescent antibody assay and ELISA are the most common methods used. Western blotting has recently been used for confirmation assays, and is normally used following a positive screening assay. Hence, some countries prefer western blotting.

2.4. Tick Classification

Approximately 896 species of ticks are known to occur worldwide, and are classified into three families: Ixodidae, Argasidae, and Nuttalliellidae (Guglielmone *et al*., 2010). Of these, the Ixodid ticks represent about 80%, and they are assumed to be one of the major arthropod groups that can affect both humans and animals (Cupp, 1991). Their feeding method causes hosts to experience allergic reactions, toxicosis, paralysis, irritation, and even death (Estrada-Peña, 2003; Ogrzewalska and Pinter, 2016). Ixodid ticks are vectors of several pathogens such as protozoa, spirochetes, rickettsia, and similar viruses that cause serious diseases (Jongejan and Uilenberg, 2004). Below are some common ticks that affect equids.

2.4.1. Genus *Amblyomma*

Amblyomma ticks are some of the largest. The genus *Amblyomma* contains approximately 130 species (Guglielmone *et al*., 2010), 28 of which are found in Africa. The features of *Amblyomma* are long palps and hypostomes. The basis capitulum is almost rectangular or sub-triangular. Most have a characteristic scutum with spots and lines of different colours, which differ between species. Moreover, their eyes are either convex or flattened. The spiracular plates are in the shape of a comma. Additionally, this genus has a post-anal groove and festoon on the edge of the abdomen (Voltzit, 2007).

This genus can transmit pathogens such as *Rickettsia africae*, *Ri. rickettsia* and *Ehrlichia ruminantium* , which are important causes of medical and veterinary diseases (Petney *et al*., 1987; Kelly *et al*., 1992; Berrada *et al*., 2011).

2.4.2. Genus *Dermacentor*

Dermacentor ticks are medium-sized. The genus *Dermacentor* has approximately 41 species (Guglielmone *et al*., 2010), only a few of which can be found in Africa. These ticks have short mouthparts and rectangular basis capitulum. The scutum of *Dermacentor* is clear and ornamental with light-colored marble patterns. This genus has festoons on the edge of the abdomen. The eyes are positioned at the back and around the second pair of legs. On the female dorsal surface, there are wide, oval, and porous sites (Brinton *et al*., 1965). *Dermacentor* spp. can transmit rickettsiae, other bacteria, and viruses(De La Fuente *et al*., 2008).

2.4.3. Genus *Rhipicephalus*

Rhipicephalus ticks are medium-sized. Currently the genus *Rhipicephalus* includes 82 species (Guglielmone *et al*., 2010). This tick has short mouthparts and palps. The basis capitulum is typically hexagonal and their eyes are flat to slightly convex. Males have festoons and large spiracular and ventral plates. This genus can transmit

rickettsiae, anaplasmosis, bacteria, viruses, and protozoans (De La Fuente *et al*., 2008).

2.4.4. Genus *Boophilus*

Boophilus ticks are among the smallest types of tick. The genus *Boophilus* currently includes approximately 5 species (Guglielmone *et al*., 2010). Some of these species are included in the genus *Rhipicephalus*. Thus, there are not many morphological differences between *Boophilus* and *Rhipicephalus*. Its mouthparts and palps are short, and the basis capitulum has angular lateral margins. The scutum of *Boophilus* is ornamental, and the spiracular plates are large. Eyes are present, however, they are not distinct. Compared to the genus *Rhipicephalus*, the genus *Boophilus* does not have a festoon. This genus can transmit bacteria, viruses, and protozoans (De La Fuente *et al*., 2008).

2.4.5. Genus *Hyalomma*

Hyalomma is one of the largest genera of ticks, similar to *Amblyomma*. Currently, the genus *Hyalomma* includes approximately 27 species (Guglielmone *et al*., 2010). *Hyalomma* ticks are also known as bont-legged ticks owing to their striped legs. The key features of *Hyalomma* are the genital aperture and mouth parts. Its dorsal appearance is hexagonal, and its surface also has festoons. In addition, the spiracular

plates have tail-like protrusions.

Hyalomma ticks are vectors for several pathogen-like viruses and *Theileria* sp. Thus, they are important arthropods from medical and veterinary perspectives (Heisch *et al*., 1962; Taboada and Merchant, 1991; Aktas *et al*., 2004; Shyma *et al*., 2012). They are also vectors of the Crimean-Congo haemorrhagic fever virus (Bente *et al*., 2013).

2.4.6. Genus *Haemaphysalis*

Haemaphysalis ticks are among the smallest ticks. Currently, the genus *Haemaphysalis* includes approximately 167 species (Guglielmone *et al*., 2010). These ticks have mouthparts and broad palps. The basis capitulum have straight lateral margins. Festoons are present; however, they are not easily distinguishable in females. The spiracular plates are large. *Haemaphysalis* ticks are vectors for rickettsiae, certain viruses, anaplasmosis, and *Ehrlichia* spp (Oh *et al*., 2009).

2.4.7. Genus Ixodes

Ixodes ticks are medium-sized. The genus *Ixodes* comprises approximately 243 species (Guglielmone *et al*., 2010). *Ixodes* ticks have mouthparts and long palps, and the basis capitulum has straight lateral margins. The scutum and conscutum do not have ornamentation. *Ixodes* ticks do not have eyes or festoons. The spiracular plates are large.

Ixodes ticks can transmit *Borrelia* spp., *Babesia* spp., *Ehrlichia* spp., and virus-like tick-borne encephalitis virus (De La Fuente *et al*., 2008).

2.4.8. Life Cycle of Ticks

Ixodid ticks have four stages in the life cycle; egg, larva, nymph, and adult. After the eggs hatch, every stage of development requires blood meals from a vertebrate host, such as mammals, birds, reptiles, and amphibians (Fig 3). Ticks have three types of life cycles; one-host, two-host, and three-host life cycles. The one-host life cycle is completed on one individual host. The genus *Boophilus* contains ticks with a one-host life cycle.

In the two-host life cycle, the larvae and nymph stages feed on a first host, such as a rodent or lagomorph, and at the adult stage, they move on to feed on a second host, such as an herbivore. The genus *Hyalomma* comprises ticks with a two-host life cycle tick.

In the three-host life cycle, each stage feeds on a different host. The larval stage normally attaches to small mammalian hosts such as rodents. The nymph attaches to medium-sized mammals such as hares. The adult stage attaches to larger mammals, such as herbivores, carnivores, or humans. as the genera *Amblyomma*, *Ixodes*, *Haemaphysalis*, and *Dermacentor* are examples of three-host life cycle tick.

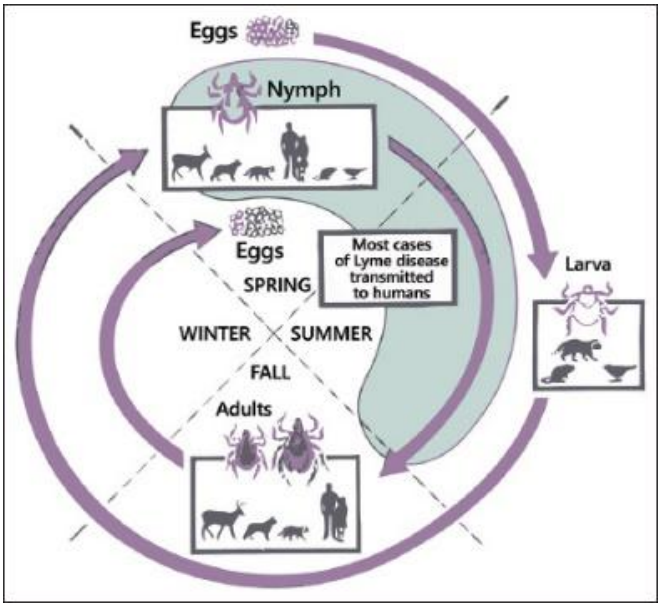


Figure 3: The general life cycle of ticks

(https://www.tickcheck.com/info/deer-tick-life-cycle-and-active-periods)

2.4.9. Ticks Reported in Zebras and Donkeys from Previous Studies

In Kenya, *R. appendiculatus*, *R. pulchellus*, *R. evertsi*, *R. microplus*, and *A. gemma* have been found on zebras (Walker, 1974; Ndeereh *et al*., 2017: Kanduma *et al*., 2020). Horak *et al*., (2021) reported that *A. hebraeum*, *Hy. truncatum*, *R. appendiculatus*, *R. decoloratus*, *R. evertsi evertsi*, and *R. simus* were found on zebras in South Africa. In Tanzania, *R. pulchellus* and *R. evertsi* have been discovered on zebras (Kim *et al*., 2018).

Walker (1974) found *R. appendiculatus*, *R. evertsi*, *R. simus*, *R. praetextatus*, *A. gemma*, and *D. rhinocerinus* on donkeys in Kenya. Lutomiah *et al*., (2014) also reported *Hy. dromedarii* on donkeys in Kenya. In Sudan, *R. evertsi*, *R. decoloratus*, *R. guilhoni*,

R. camicasi, *A. lepidum*, *Hy*. i*mpeltatum*, *Hy*. *rufipes*, *Hy*. *excavatum*, *Hy*. *dromedarii* and *Hy*. *anatolicum* have been found on donkeys (Bala *et al*., 2018). Halajian *et al*. (2018) discovered *A. hebraeum*, *R. appendiculatus*, *R. evertsi evertsi*, *R. simus*, *Rhipicephalus* spp., and *Hy*. *rufipes* on donkeys in South Africa.

2.4.10. Identification of Ticks

Tick identification is normally performed based on morphological characteristics. However, it can be difficult to identify tick species because tick bodies are often damaged when the ticks are removed from their hosts. In addition, if tick samples are poorly preserved, it is difficult to identify the tick by means of morphology (Chitimia *et al*., 2009). In the event that morphological identification is difficult, molecular techniques can be useful as alternative tick identification techniques. There are differences in DNA tick between species. For tick diversity studies, most people use the internal transaction spencer 2 gene (*ITS2*) or cytochrome c oxidase subunit 1 (*CO1*), both of which are mitochondrial markers. Other nuclear ribosomal genes include 18S rDNA, 28S rDNA, 12S rDNA, 16S rDNA, ITS1, and CO3 (Cruickshank, 2002; Dabert, 2006; Takano *et al*., 2014).

2.4.11. Tick Control

2.4.11.1. Chemical Control

The conventional method for controlling ticks involves dipping or spraying host animals with acaricides. However, there are disadvantages in using acaricides. including tick resistance and ecological pollution. The first synthetic insecticides were organochlorine insecticides, such as dichlorodiphenyltrichloroethane (DDT) and benzene hexachloride (BHC), which were used to control ticks in cattle (Whitnall *et al*., 1951). However, organochlorine insecticides such as DDT, BHC, and cyclodienes have drawbacks because they remain in the environment and accumulate in body fat (Ware and Whitacre, 2004).

2.4.11.2. Host Resistance to Ticks

Tick saliva contains bioactive substances such as vasodilators and anti-inflammatory and immunosuppressive agents (Wikel, 1999; Nuttall, 2019). These substances are injected into the host, and some host animals have an innate immune response and develop acquired immunity against tick infection. The acquired immunity of the host animals results in a reduction in the weight of ticks, a reduction in the number of ticks feeding on host animals, and inhibition of molting (Willadsen, 2004). These factors result in a decrease in tick populations.

2.4.11.3. Anti-Tick Vaccines

Anti-tick vaccines are made using a variety of antigenic materials, which comprise homogenized whole ticks, salivary glands, and tick internal organs. Tick antigens belong to two groups: naturally acquired resistance, and concealed antigens. Concealed antigens, which are immunogenic proteins, are obtained from the tick gut. γ-immunoglobulins in the blood of the host can pass through the intestinal wall and into the haemolymph of the tick (Jongejan and Uilenberg, 1994). These host immunoglobulins cause damage to the tick's gut while it is feasting on the host (Kemp *et al*., 1989).

2.5. Gastrointestinal Parasites of Equids

2.5.1. Small Strongyle (*Cyathostoma*)

Cyathostomins are the most common gastrointestinal parasites that infect both horses and ponies. They are capable of causing clinical diseases in equids. The larva 3 (L3) stage, which is the infective stage, is ingested by the equid species during grazing. Once ingested by host animals, L3 passes through the small intestine, removes the outer sheath, and initiates further development of the parasites. The new eggs and adult worms are likely to be passed in the faeces after 5–6 weeks. The symptoms of cyathostomins infection include diarrhoea, weight loss, colic (Krecek and Guthrie,

1999), and anaemia (Matthee *et al*., 2002).

2.5.2. Large Strongyle (*Strongylus*)

Donkeys are hosts for the large strongyle (*Strongylus*) species. *S. vulgaris* migrates into the blood vessels of the small intestine, where it takes approximately 6 to 7 months to make its way into the donkey cells. *S. edentates* and *S. equines* migrate into the liver, where they take 8 to 11 months to arrive at the donkey cells. The life cycle and epidemiology of the large strongyle is similar to that of the cyathostomins. The symptoms of large strongyle infection are weight loss, diarrhoea, and colic (Krecek and Guthrie, 1999).

2.5.3. *Parascaris equorum*

Parascaris equorum is a small intestinal nematode that does not usually affect donkey health. The life cycle of *Parascaris* sp. resembles that of strongyles. However, the infective stage involves a thick-walled egg with an infective larva. This egg can remain alive in that environment for up to 10 years. The eggs are ingested when equids are grazing, and they migrate to the large intestine and other organs, such as the liver, pancreas, and arteries. This migration is associated with colic and death of equids.

2.5.4. *Oxyuris equi*

Oxyuris equi is a nematode and is not a common species, like the cyathostomins (Upjohn *et al*., 2010). Parasites are normally observed as adult worms when they pass through faeces. Thus, parasites can be detected in samples from the perineal region. The life cycle of *O. equi* is similar to that of *Parascaris* spp. The infective stage of *O. equi* is an egg containing at third-stage larva, with the host being infected after ingestion. The larvae (L3) hatch in the small intestine, enter the large intestine, and enter the mucosal crypts of the caecum and colon. This can cause damage to the perineal region. However, donkeys do not appear to be affected.

2.5.5. *Dictyocaulus arnfieldi*

Dictyocaulus arnfieldi is commonly found in donkeys. The life cycle of *Dictyocaulus* is similar to that of *Strongylus*. L3 is the infection stage, when host animals become infected with *Dictyocaulus* sp. during grazing. These larvae migrate through the intestinal wall, shed to L4 at the lymph nodes, reach the bronchioles, and shed to L5 or pre-adults. L5 larvae move to the trachea and bronchi and complete their growth into adults. Adult females then lay eggs that are transported to the pharynx in respiratory secretions. From the pharynx, these eggs are directly coughed out or swallowed. When an egg is swallowed, L1 is released into the intestine and excreted

from the faeces. In the environment, L1 develops to infective stage L3 in approximately 1 week. Donkeys normally do not exhibit any symptoms unless the number of parasites is high. If disease symptoms occur in donkeys, they likely arise in geriatric or immunocompromised animals.

2.5.6. *Anoplocephala perfoliata*

Anoplocephala perfoliata is a common cestode with an indirect life cycle (vector-borne parasites) in equid species. This parasite is weak in dry environments. However, the parasite can survive for months, even during the cold winter season. The intermediate host, the oribatid mite, ingests eggs. In the mites, the eggs hatch and develop to the cysticercoid stage, which is the stage of infection for the definitive host. After ingesting contaminated mites during grazing, the definitive host becomes infected. The mites are digested, release cysts attached to the intestinal wall, and grow into adult tapeworms within approximately 6 weeks.

2.5.7. *Fasciola hepatica*

Fasciola hepatica is a common trematode affecting all grazing animals. Adult flukes are found in the bile duct. However, the disease symptoms are not normally observed in donkeys. When parasite eggs reach the ground, they need to be moist to

mature and hatch into miracidium. Miracidia leave the faeces and move to the intermediate host, the snail genus *Lymnaea*. Miracidium penetrates the snail body and undergoes hundreds of clonal replications. Subsequently, the cercaria leaves the snail and parasitizes the herb as metacercaria, which is the infective stage. Metacercariae can survive for up to several months. However, some die at high or low temperatures, or in dry environment. Equid grazing pastures become contaminated with metacercariae, which become encysted on the herbage. The juvenile stage penetrates the intestinal epithelium after ingestion, crosses the peritoneum, and then penetrates the liver capsule. The parasite then moves to the liver parenchyma. Some may reach patency, rise to maturity in bile ducts, and lay eggs which are excreted in the faeces.

2.5.8. Gastrointestinal Parasites Reported in Zebras and Donkeys from Previous Studies

Lewa *et al*. (1999) reported an association between the number of strongyle eggs and haematological changes in donkeys. Jajere *et al*., (2016) showed that 98.3% of donkeys were infected with gastrointestinal parasites, 78.3% were infected with strongyle, 40.3% with *P. equorum*, and 17.5% with O. *equi*. In Sudan, Ismail *et al*., (2016) reported that 97.8% of donkeys were infected with gastrointestinal parasites, 40.2% with *Habronema* sp., 30.4% with *Trichostrongylus axei*, 18.5% with *P. equorum*,

4.35% with *A. perfoliata*, 8.7% with *Gastrodiscus aegyptiacus*, 84% with large strongyle, 72% with small strongyle, and 1.1% with *O. equi*.

Wambwa *et al*. (2004) studied and compared the infection of gastrointestinal parasites between domesticated and free-ranging Burchell's zebra (*Equus burchelli*). Free-ranging zebras had a heavier load of parasites than ranched zebras, including the famiies Atractidae, Strongylidae, Spiruridae, Oxyuridae, Setariidae, Anoplocephalidae, Ascaridae, and Gasterophilidae.

2.5.9. Diagnosis of Gastrointestinal Parasites

The methods for the identification of gastrointestinal parasites include direct smear, agar plates, flotation, sedimentation, Kato-Katz, Harada Mori, and Baermann methods. The sugar flotation method is more effective in detecting gastrointestinal eggs than other flotation methods (Dryden *et al*., 2005). Centrifugation methods, such as sedimentation and flotation, can recover more eggs than other methods. The McMaster method is a quantitative method for faecal egg count. However, these methods are not particularly useful for identifying species. The culture method has proven to be the most useful identifying species of adult worms.

The molecular technique (PCR) is more effective in detecting specific species than microscopic methods and has been further developed in recent years.

2.5.10. Epidemiology of Gastrointestinal Parasites

Locations with high rainfall generally have more gastrointestinal parasite problems than other areas (Williams *et al*., 1983). Considering the distribution of gastrointestinal parasites, rainfall is recognized as more important than other factors (Hutchinson *et al*., 1989; Hansen and Perry, 1994). For example, in hot and sunny climates, faeces dry easily, causing parasites to grow slowly or stop developing entirely (Rossanigo and Gruner, 1995). Humidity and moisture are important factors for parasite growth and development (Charlier *et al*., 2020). Thus, climate and temperature affect the development and distribution of gastrointestinal parasites.

Young equids have a higher prevalence of gastrointestinal parasites than other age groups (Regassa and Yimer, 2013; Tesfu *et al*., 2014; Jajere *et al*., 2016). Jajere *et al*. (2016) suggested the possibility that young equids acquire adequate adaptive immunity to gastrointestinal parasites more slowly than other age groups. Hence, young equids are at higher risk of severe gastrointestinal parasite infection. Moreover, body condition also affects the infection rate of gastrointestinal parasites. If the body condition is poor, parasitic infections in donkeys occur at a higher rate (Ayele *et al*., 2006; Tesfu *et al*., 2014; Ibrahim *et al*., 2011; Jajere *et al*., 2016).

CHAPTER THREE

MATERIALS AND METHODS

3.1. Study Area Description

This study was conducted in and around The Amboseli National Park (Figure 4), which is located in the northern part of Mt. Kilimanjaro along the Kenya-Tanzania border. The area is characterized by a habitat that extends from wetlands to woodlands, grasslands, and thorn bushes.

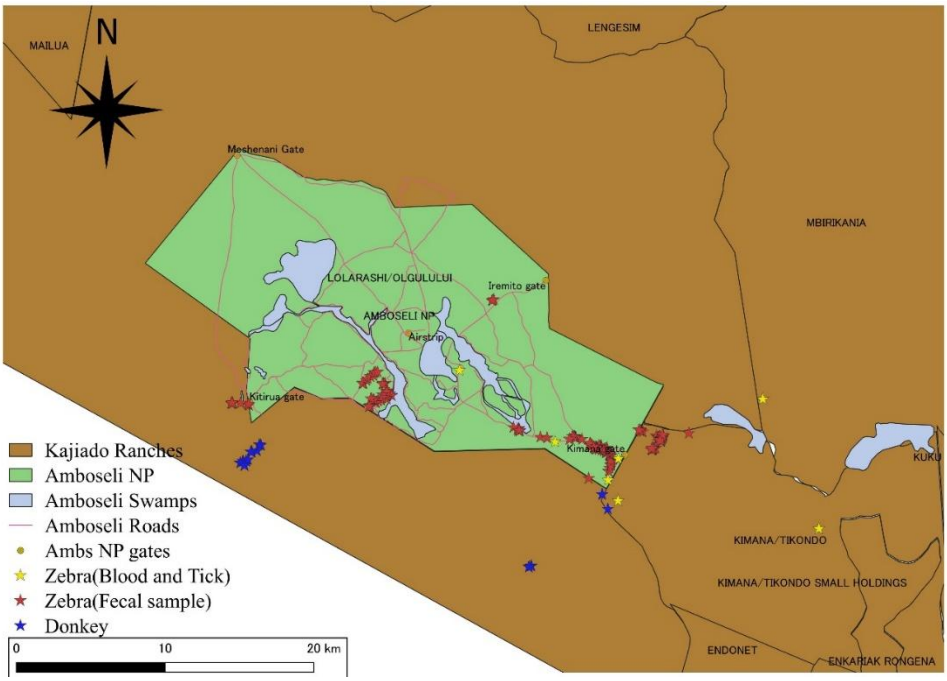


Figure 4: Map of the Amboseli National Park and its adjacent areas showing the sampling sites

★ sampling points where blood samples from zebras were collected.

★ sampling points where faecal samples from zebras were collected.

★ sampling points where faecal, blood and tick samples from donkeys were collected.

3.2. Study Design

The study was undertaken between November 2019 to November 2020 and used a cross-sectional study design.

3.2.1. Study Approval

This study was approved by the Biodiversity, Research and Planning Department of the Kenya Wildlife Service, under approved number KWS/BRP/5001 (Annex 1).

3.2.2. Sample Size Calculation

Assuming the prevalence of parasite of 86% and the precision error of 5% sample size was calculated using the formula from Martin et al, 1987; n=$z_\alpha^2\, pq/L^2$ where: n = Sample size: z_α = Confidence level 95% (1.96): p =assumed 86% prevalence of parasites: q=1- p: L = The precision error (5%). Hence n $(1.96)^2$ (0.86) (0.14) / $(0.05)^2$ = 185 samples inclusive of ticks, blood, and faecal samples.

3.3. Collection of Blood Samples from Zebras and Donkeys

The blood samples from zebras were collected by when the Kenya Wildlife Service scheduled management activities such as clinical treatment. Donkey owners were required to give verbal consent before the donkeys were sampled. Donkeys were

restrained manually by the owners and blood was collected into 5 ml labeled EDTA tubes from the jugular vein using a 18G syringe needle, then transported back to the laboratory in a cool box packed with ice bricks.

3.3.1. DNA Extraction from Blood Samples

Total genomic DNA was extracted and purified from zebra and donkey blood sample using the Qiamp DNeasy blood and tissue kit (QIAGEN, Germany) according to the manufacture's instruction. Briefly, 200µl of anticoagulated blood added with 20µl of proteinase K in a 1.5ml Eppendorf tube. Two hundred micro-litter (200µl) of Lysis buffer AL was added to the mixture, vortexed and incubated at 56˚C for 10 minutes. Two hundred micro-litter (200µl) absolute ethanol was then added to the mixture and vortexed. After that, the mixture was pipetted into the DNeasy Mini spin column. The column was then centrifuged at 8000rpm for 1 minute and the flow-through discarded. After that, the bound DNA in the column was washed with 500µl of Buffer AW1. The column was then centrifuged for 1 minute at 8000rpm. A second wash with 500µl of Buffer AW2 was followed by centrifuging for 3 minutes at 14000rpm to dry the DNeasy membrane. Fifty micro-litter (50µl) of elution Buffer AE eluted bound DNA into a clean 1.5ml tube, and the bound DNA was stored at -20˚C until further use.

3.3.2. Polymerase Chain Reaction (PCR) Amplification of *Theileria*/*Babesia* 18s rRNA gene fragment

A nested PCR assay was done targeting 450-500bp of the 18sRNA gene fragment using primers previously described by Hawkins *et al*., (2015). The primer sequences are indicated Table 1. The primary PCR reaction was done in a 10µl total reaction volume containing the following: 5µl of HotStarTaq Master Mix (QIAGEN, Hidden, Germany), 2µl of the template DNA, 0.5µl of each forward and reverse primers, and 2µl of sterile reagent PCR water. The amplification was performed using a T 100 thermocycler (Bio-Rad) under reaction conditions previous described by Hawkins *et al*., (2015). The cycling conditions were an initial denaturation at 95℃ for 5 minutes, followed by 30 cycles of denaturation for 30 seconds at 95℃, annealing at 55℃ for 30 seconds, and extension at 72℃ for 60 seconds. A final extension was then done at 72℃ for 9 minutes.

The secondary PCR reaction was done in a 25µl total reaction volume containing 12.5µl HotStarTaq Master Mix (QIAGEN, Hidden, Germany), 2µl of the primary PCR product, 0.5µl of each forward and reverse primers, and 9.5µl of sterile reagent PCR water. The cycling conditions were as described for the primary PCR.

Table 1: Primer sequence of *Theileria*/*Babesia* and *Ehrlichia*/*Anaplasma* in this study

	Pathogens	Primaer	Start	End	Sequence	Reference	Remark
First	*Theileria* /*Babesia*	ILO-9029			5'CGGTAATTCCAGCTCCAATAGCGT-3'	Hawkins et al. 2015	Forward
		ILO-9030			5'-TTTCTCTCAAAGGTGCTGAAGGAGT-3'		Reverse
	Ehrlichia /*Anaplasma*	EF1	1109	1126	5'-CTGAYGGTATGCAGTTTG-3'	Park et al. 2005	Forward
		ER2	1511	1495	5'-AYRYYTTTAGCAGTACC-3'		Reverse
Second	*Theileria* /*Babesia*	ILO-9029			5'CGGTAATTCCAGCTCCAATAGCGT-3'	Hawkins et al. 2015	Forward
		ILO-7782			5'-AACTGACGACCTCCAATCTCTAGTC-3'		Reverse
	Ehrlichia /*Anaplasma*	EF3	1114	1130	5'-GGTATGCAGTTTGAYCG-3'	Park et al. 2005	Forward
		ER4	1400	1384	5'-TCTTTTCTYCTRTCACC-3'		Reverse

3.3.3. Polymerase Chain Reaction (PCR) Amplification of *Ehrlichia*/*Anaplasma* 16s rRNA gene fragment

A nested PCR assay targeting a 300bp fragment of the 16s RNA gene fragment was done using primers previously described by Park *et al*., (2005) (Table 1). The primary reaction mix was as described for the *Theileria*/*Babesia* (section 3.3.2) only that OneTaq1 Quick-Load 2X Master Mix with Standard Buffer (New England Bio-labs-NEB, Massachusetts, USA) was used. The secondary PCR reaction mix was as described for the *Theileria*/*Babesia* (Section 3.3.2). Amplification was performed using a T 100 thermocycler (Bio-Rad) under reaction conditions previous described by Park et al., (2005). The cycling conditions were an initial denaturation at 94℃ for 5 minutes, followed by 20 cycles of a denaturation for 20 seconds at 94℃, annealing at 50℃ for 20 seconds, and at 68℃ extension for 30 seconds. A final extension was then done at 68℃ for 5 minutes

3.3.4. Agarose Gel Electrophoresis of Amplified PCR products

Five microliters of the amplified PCR products were resolved on 1.5% agarose gel by sub marine gel electrophoresis using 1 x TAE as the running buffer. A DNA ladder (100bp marker) was a run alongside the amplified products at 90 volts for 1 hour.

The gel was visualized under UV light following the staining with ethidium bromide.

3.3.5. Sequencing of 18s and 16s Amplified PCR products

All positive PCR products was submitted to Macrogen Inc., (https://dna.macrogen-europe.com/eng/) The Netherlands and were sequenced by the Sanger sequencing technology using both the forward and reverse primers.

3.4. Collection and Identification of Tick Samples from Zebras and Donkeys

Tick samples were collected from 3 zebras and 38 donkeys by checking the ear or body surface. The ticks were collected from only three zebras because of whether there was no ticks on other zebras. The ticks were collected from both zebras and donkeys, and preserved in 70% ethanol until identification was performed. Ticks were examined for morphological characteristics under a stereomicroscope. Life stage and sex were recorded using standard descriptions (Walker, *et al*., 2003; Horak *et al*., 2018).

3.5. Collection of Faecal Samples from Zebras and Donkeys

Zebras and donkeys were followed to allow identification and faecal sample collected from the ground as soon as defecated (Figure 5). Faecal samples were collected from total 186 zebras and 43 donkeys and approximately 50g of faecal sample collected was preserved by fixing in 10% formalin then transported to the laboratory for

further processing.

Figure 5: The representative of faecal collection (① defecating faecal, ② the faecal of zebra, ③ collecting faecal sample)

3.5.1. The Sugar Flotation Technique for Identification of Gastrointestinal Parasites from Faecal Samples

A flotation solution was made by mixing 454g of table sugar and 355ml of distilled water. The mixture was warmed over until all the sugar crystals were dissolved then left to cool the room temperature. Four grams of the faecal samples which were fixed in formalin were emulsified in 12ml of tap water. The mixture was filtered through a tea strainer into a 15 ml plastic centrifuge tube. The sample was refilled with tap water up to the 14 ml mark and centrifuged at 1500rpm for 10 minutes. The suspension was poured off and refilled the half of the volume of the tube with the flotation solution then mixed using a stir bar. The tube was then filled with the flotation solution until it formed a meniscus. A coverslip was then gently place on top of the meniscus and the tubes left to stand for 2 hours. The tube was then centrifuged at 1500 rpm for 10 minutes and the coverslip then gently placed on to microscope slide and examined under a microscope

(Leica DM500, Leica microsystems, UK) at X10 and X40 magnification. Photo images and dimensions were taken using the LAZ EZ micro-imaging software version 2.0 (Leica microsystems, UK) attached to the camera.

3.5.2. The Modified Sedimentation Technique for Identification of Gastrointestinal Parasites from Faecal Samples

Four grams of the faecal samples were emulsified in 45ml of tap water. The mixture was then filtrated through a tea strainer into a 50ml plastic centrifuge tube then left to stand for 10 minutes. The suspension was gently poured off and the sediment resuspended in 45ml of tap water and left to stand for another 10 minutes. This process of resuspension and decanting was continued three times or until the suspension became clear. The sediment was then removed and placed onto a glass slide covered with a coverslip then observed under the microscope as described in section 3.5.1.

3.6. Data Analysis

The prevalence of parasites was calculated as the percentage of positive samples in comparison to the total number of examined samples. Data were analysed by chi-square test using R software (version 4. 1. 0.) and the significance level was at $p<0.05$.

Consensus sequence of the 18s and 16s regions were separately assembled for

each sample using DNAStar software (DNAStar, Inc., Madison, WI). The sequences were manually edited and trimmed to remove low quality reads. Using BLASTn algorithm (https://blast.ncbi.nlm.nih.gov/Blast.cgi), molecular identification of species was detected by comparing with sequence from this study with those available in NCBI database. The DNA sequences for each gene were then aligned using Mega 7 software (https://www.megasoft.net/) by applying ClustalW algorithm.

CHAPTER FOUR

RESULTS

4.1. Tick Infestation in Zebras and Donkeys

A total of 367 adult and 2 immature (nymphs) ixodid ticks were collected from three out of fifteen zebras, giving an overall infestation prevalence of 20%. Total number of ticks collected from donkeys were 64 adults and a single nymph. These ticks were collected from 37 out of the 79 donkeys were giving an overall prevalence of 47% (Table 2).

Three tick genera were identified and included *Rhipicephalus* (88%), *Amblyomma* (7%) and *Hyalomma* (5%) from zebra (Figure 6). *R. pulchellus* was the most abundant tick species in zebra and comprised 78% of the total ticks collected, this followed by *R. evertsi* (7%), *A. gemma* (7%), *R. praetextatus* (2%), *Hy. albiparmatum* (2%), *Hy. impeltatum* (0.27%), and *Hy. rufipes* (0.27%). *Rhipicephalus* species were the only ticks found on donkeys with the most abundant species being *R. pulchellus* (98%) followed by *R. praetextatus* (2%).

Table 2: The species and prevalence of tick infestation on zebras and donkeys in the Amboseli National Park and adjacent areas

Animal	Tick species		Prevalence(%)	No. of zebra	Nymph	Female	Male
Zebra (n=15)	*Rhipicephalus*	*evertsi*	20	3	0	3	2
	Rhipicephalus	*pulchellus*	20	3	0	3	3
	Rhipicephalus	*praetextatus*	13	2	1	1	2
	Hyalomma	*albiparmatum*	20	3	0	3	3
	Hyalomma	*impeltatum*	7	1	0	0	1
	Hyalomma	*rufipes*	7	1	0	0	1
	Amblyomma	*gemma*	20	3	0	2	3
Donkey (n=74)	*Rhipicephalus*	*pulchellus*	46	36	1	25	23
	Rhipicephalus	*praetextatus*	1	1	0	0	1

Figure 6: The representative images of ticks obtained from zebras and donkeys

a: *Rhipicephalus pulchellus* male,

b: *R. pulchellus* female,

c: *R. evertsi* female,

d: *Amblyomma gemma* (Left: Male, Right: Female)

4.2. Tick-Borne Haemoparasites in Zebras and Donkeys

Primers targeting the 18s rRNA gene of *Theileria*/*Babesia* were used to amplify a 450bp fragment using genomic DNA extracted from both zebra and donkey blood samples (Figure 7, Panel A). Amplifications were successfully obtained from 14 out of 15 zebra and 73 out of 79 donkey samples, representing a prevalence 93% and 92% respectively. Molecular characterization was conducted by sequencing of the 18s rRNA PCR products and clean edited sequences obtained from 13 zebra and 66 donkey samples. Nucleotide BASTN analysis of the 18s rRNA edited sequence from both zebra and donkey showed a high identity value with *Theileria equi* sequences (Accession Number: KF97074, KX148604) available at GeneBank database, with the percentage similarity ranging from 97% to 100% (Figure 8).

Analysis of the donkey 18s rRNA gene sequences revealed 9 haplotypes of *T. equi* (Figure 9), while all the 18s rRNA zebra sequence were 100% identical. All the 9 haplotypes generated from this study were deposited under the GeneBank Accession Numbers OL587938 - OL587946.

The 16s rRNA gene primers were used to amplify a 300bp fragment from genomic DNA extracted from zebra and donkey blood samples. However, no *Ehrlichia*/*Anaplasma* infections were detected in all the zebra samples (Figure 7, Panel

B), but amplifications representing *Ehrlichia*/*Anaplasma* infections were obtained from 5 donkey samples. Analysis of the 4 edited 16s rRNA gene sequences showed a 100% sequence conservation and a similarity of 88% with the sequence of *Anaplasma* cf. *platys* (Accession Number: KY709325) available in GeneBank database (Figure 10).

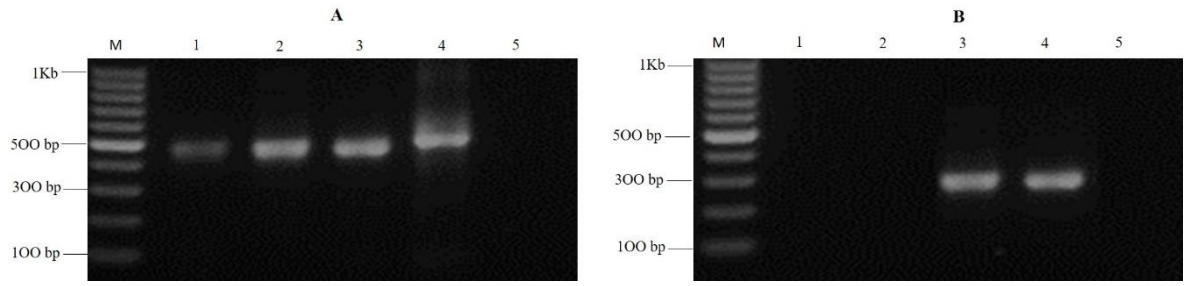


Figure 7: Representative agarose gel images of 18s rRNA and 16s rRNA of amplified gene fragment

M is 100bp molecular weight marker (Thermo Fisher Scientific)

Lane 1 and 2 represent amplified product from zebras while lane 3 and 4 are from donkey samples

Lane 5 is the Negative control

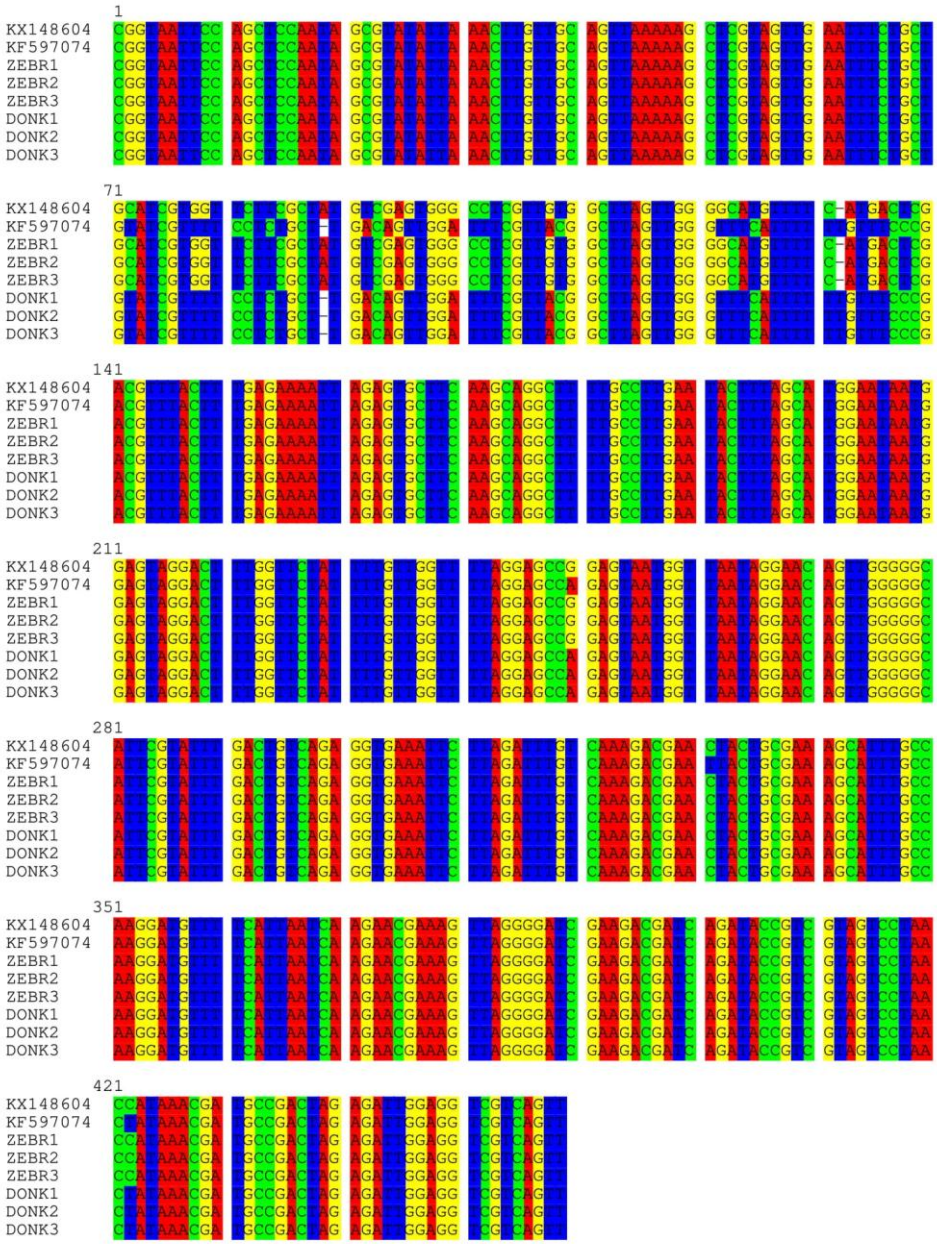


Figure 8: Multiple nucleotide analysis of 18s rRNA sequence of *Theileria equi* from representative zebra and donkey samples

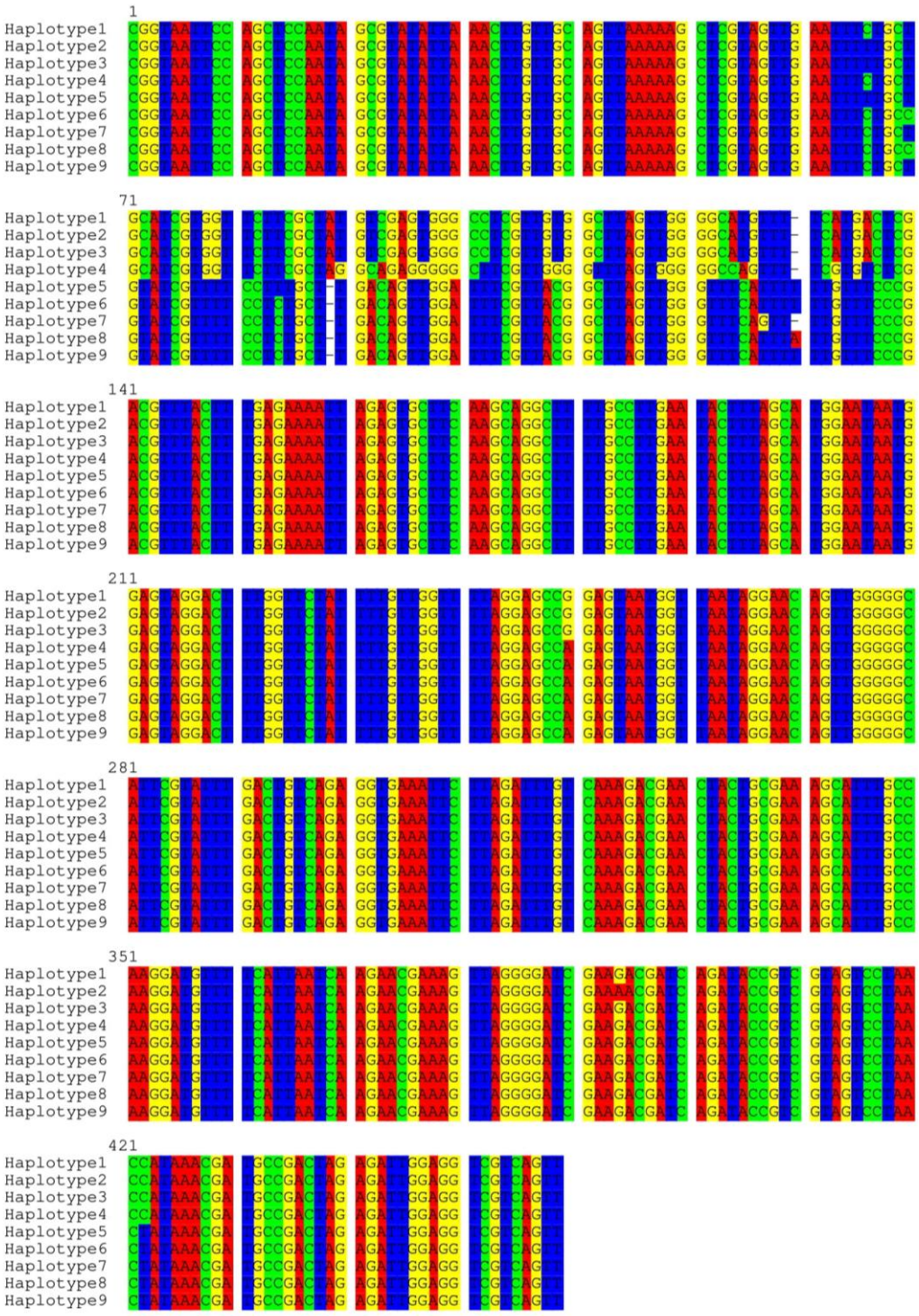


Figure 9: Multiple nucleotide analysis of *Theileria equi* of 18s rRNA haplotypes

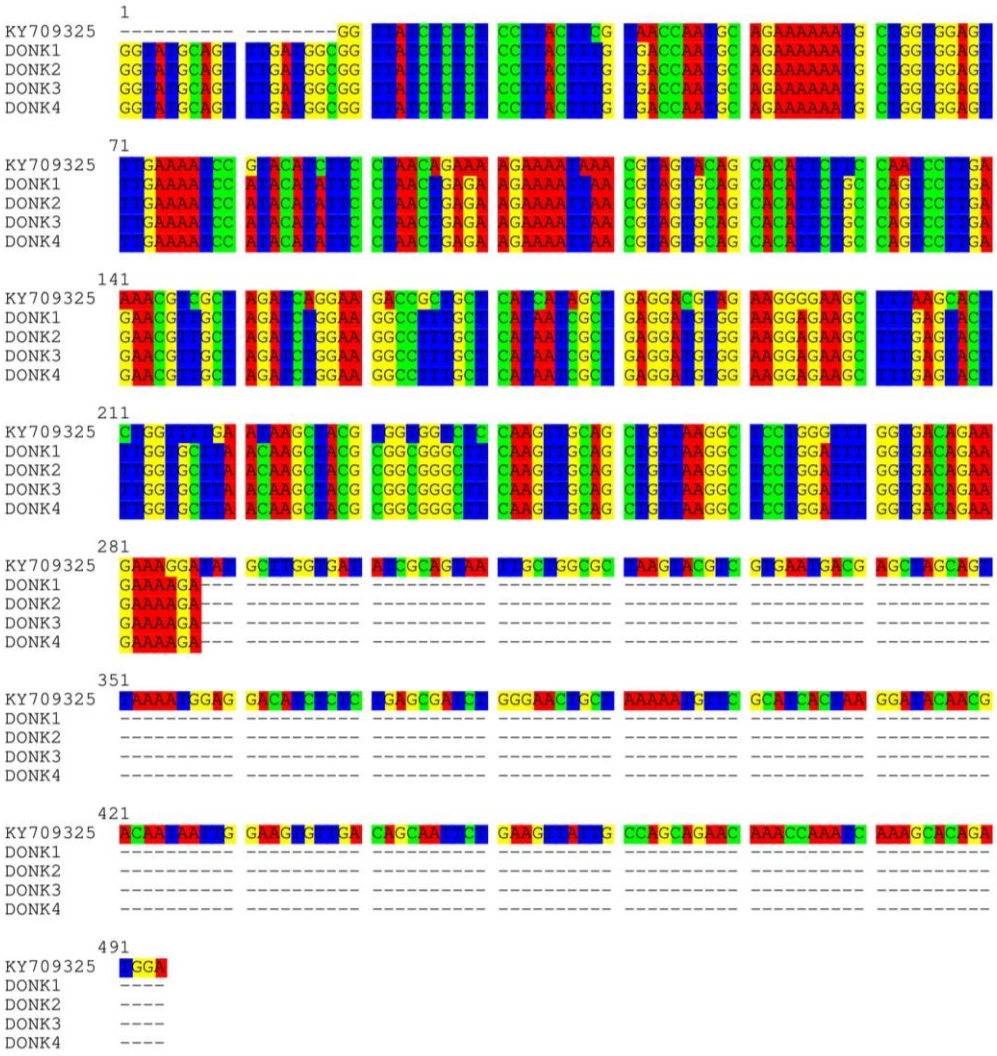


Figure 10: Multiple nucleotide analysis of 16s rRNA sequence of *Anaplasma* cf. *platys* obtained from donkey

4.3. Gastrointestinal Parasites in Zebras

A total of 10 genera/families of gastrointestinal parasites were detected from 186 faecal samples collected from zebra. Only one sample was negative of parasites, with the remaining 185 samples having single or mixed infections.

The sugar flotation technique detected 7 genera/families of gastrointestinal parasites from 185 faecal samples. These included *Anoplocephala* sp. (27%), *Parascaris* sp. (6%), strongyle (99%), *Trichostrongylus* sp. (6%), *Dictyocaulus* sp. (1%), *Oxyuris* sp. (1%), *Habronema* sp. (1%), and *Dictyocaulus* sp. larva (3%) from zebra faecal samples (Table 3, Fig 11).

The modified sedimentation technique detected 8 genera/families of gastrointestinal parasites which included Ciliates (94%), *Anoplocephala* sp. (3%), *Parascaris* sp. (2%), *Strongylus edentates* (1%), strongyle (98%), *Ttrichostrongylus* sp. (12%), *Dictyocaulus* sp. (1%), trematode (2%), and *Dictyocaulus* sp. larva (2%) (Table 3, Fig 11).

The sugar flotation technique detected significantly more *Anoplocephala* sp. *Parascaris* sp., strongyle, and *Trichostrongylus* sp. compared to the modified sedimentation technique ($p<0.05$).

Table 3: Prevalence of gastrointestinal parasites in zebras and donkeys at the Amboseli National Park

	GIP	Sugar Flotation technique (Prevalence %)	Modified Sedimentation technique (Prevalence %)
Zebra (Total:186)	Oocyst	35	1
	Ciliates	0	94
	Anoplocephala sp.	27	3
	Parascaris sp.	6	2
	Strongylus edentatus	0	1
	Strongyle	99	98
	Trichostrongylus sp	6	12
	Dictyocaulus sp	1	1
	Trematode	0	2
	Oxyuris sp.	1	0
	Habronema sp.	1	0
	Dictyocaulus sp. Larva	3	2
	Larva	26	9
	Strongyloides sp.	0	0
Donkey (Total:56)	Oocyst	2	0
	Ciliates	29	84
	Anoplocephala sp	2	0
	Parascaris sp	16	13
	Strongylus edentatus	0	0
	Strongyle	95	82
	Trichostrongylus sp	48	36
	Dictyocaulus sp	0	0
	Trematode	0	0
	Oxyuris sp.	0	2
	Habronema sp.	0	0
	Dictyocaulus sp. Larva	2	0
	Larva	11	5
	Strongyloides sp.	2	11

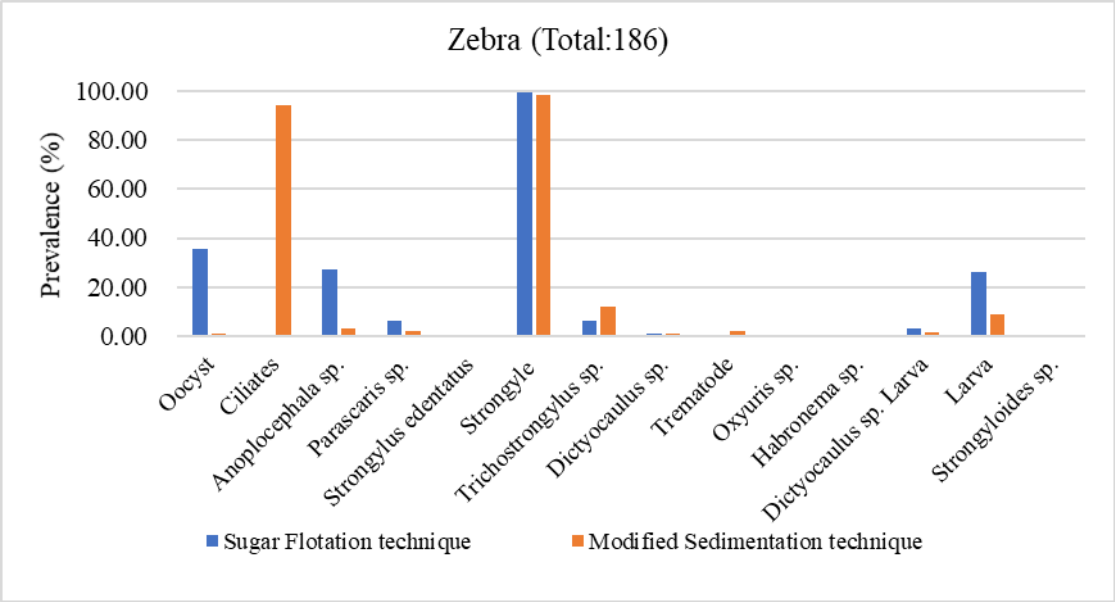


Figure 11: Prevalence of gastrointestinal parasites in zebras at the Amboseli National Park

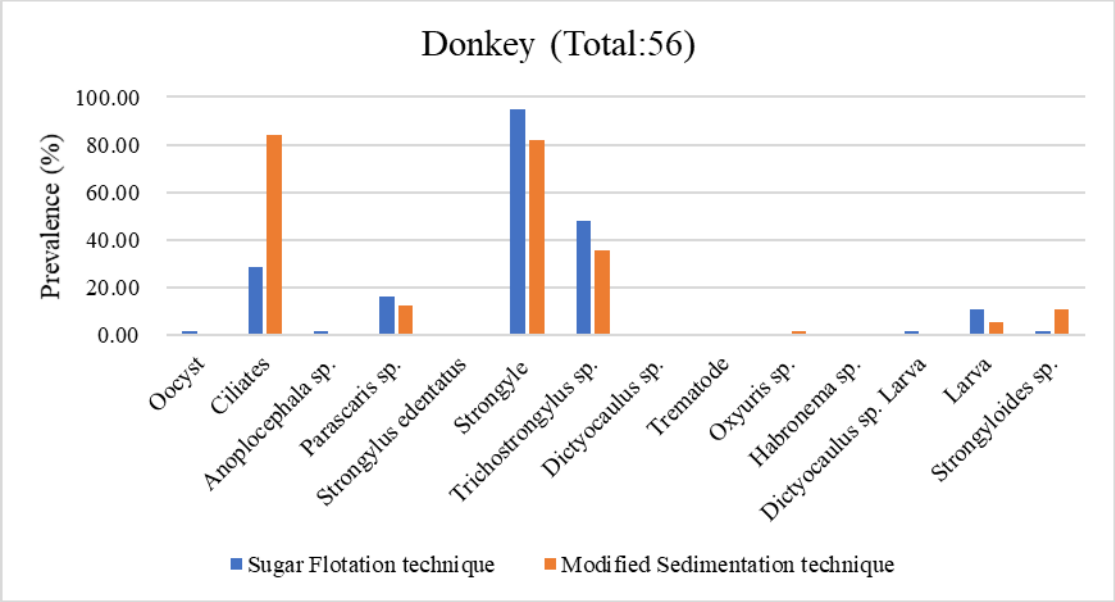


Figure 12: Prevalence of gastrointestinal parasites in donkeys at the Amboseli National Park

4.4. Gastrointestinal Parasites in Donkeys

A total of 8 genera/families of gastrointestinal parasites were detected from 65 faecal samples collected from donkey (Table 3, Fig 12). The sugar flotation technique detected 7 genera/families which included ciliates (29%), *Anoplocephala* sp. (2%), *Parascaris* sp. (16%), strongyle (95%), *Trichostrongylus* sp. (48%), *Strongyloides* sp. (2%), and *Dictyocaulus* sp. larva (2%). The modified sedimentation technique detected 6 genera/families of gastrointestinal parasites which included ciliates (84%), *Parascaris* sp. (13%), strongyle (82%), *Trichostrongylus* sp. (36%), *Oxyuris* sp. (2%), and *Strongyloides* sp. (11%). Significantly more *Parascaris* sp. and strongyle were detected using the sugar flotation technique compared to the modified sedimentation technique ($p<0.05$).

Representative images of gastrointestinal parasites obtained from zebra and donkey faecal samples are showing Figure 13.

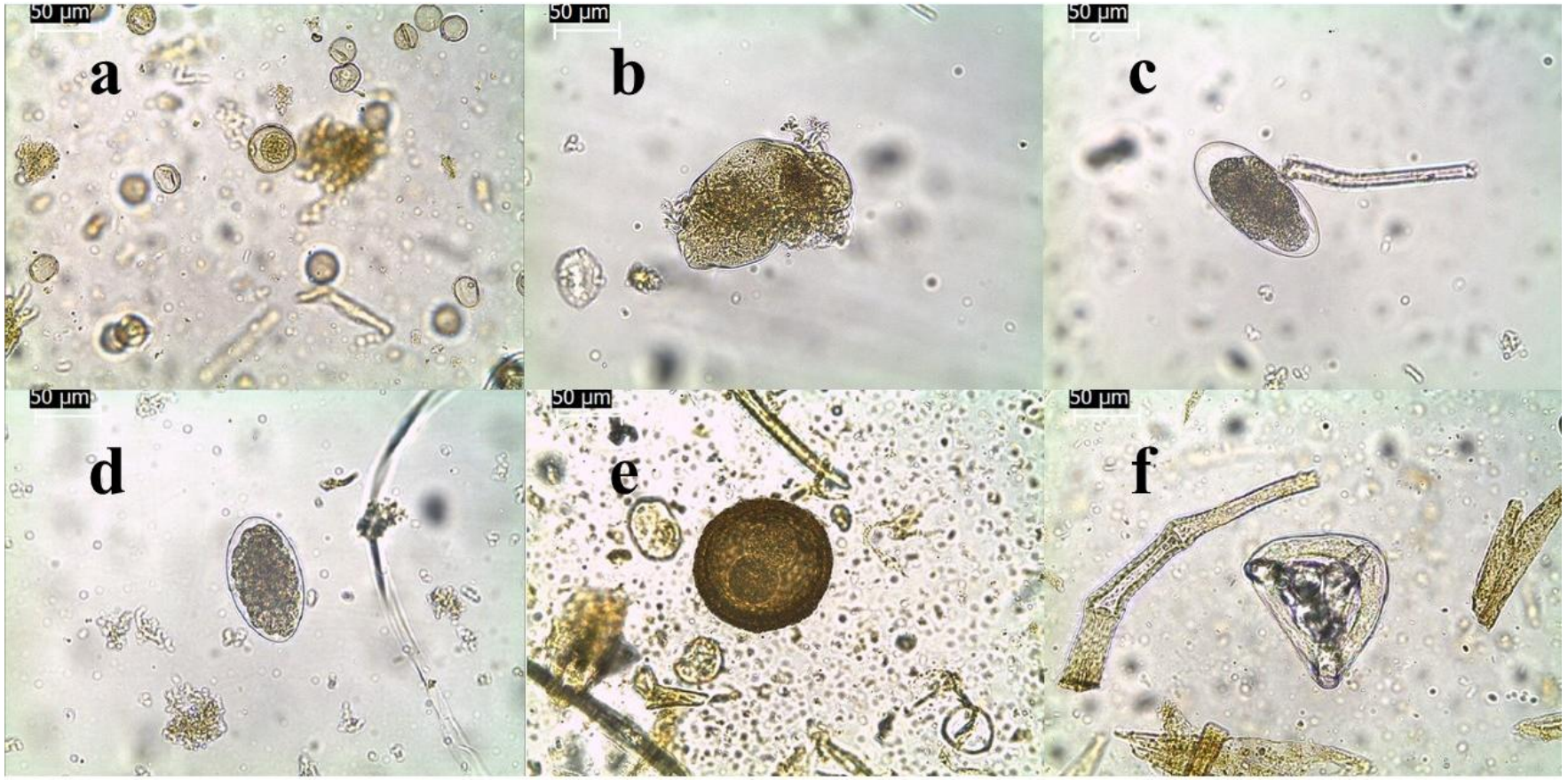
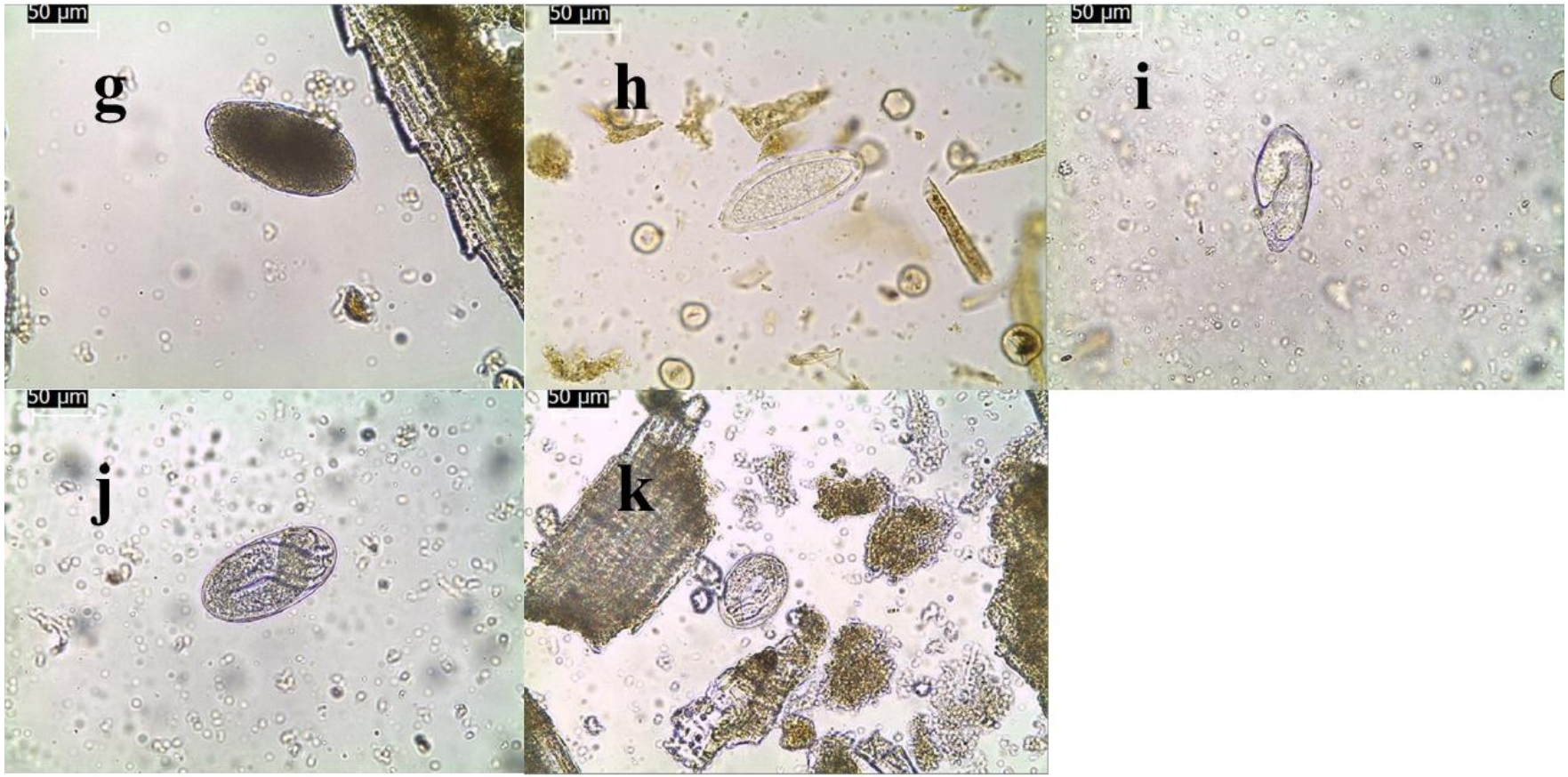


Figure 13: Representative images of gastrointestinal parasites isolated from zebras and donkeys

(a: oocyst, b: ciliate, c: strongyle, d: *Trichostrongylus* sp., e: *Parascaris* sp., f: *Anoplocephala* sp.)



(g: trematode, h: *Oxyuris* sp., i: *Habronema* sp., j: *Dictyocaulus* sp., k: *Strongyloides* sp.)

CHAPTER FIVE
DISCUSSION, CONCLUSION AND RECOMMENDATION

5.1. Discussion

This study identified the tick species infesting zebras and donkeys at the Amboseli National Park and adjacent areas. The ticks, *R. pulchellus*, *R. praetextatus*, *R. evertsi*, *Hy. albiparmatum*, *Hy. impeltatum*, *Hy. Rufipes*, and *A. gemma* were found on zebras; meanwhile, *R. pulchellus* and *R. praetextatus* were found on donkeys. On both zebras and donkeys, the most abundant species was *R. pulchellus* (also referred to as "zebra tick"). These findings support those of previous studies reporting *R. pulchellus* on zebras and donkeys (Cumming, 1998; Walker *et al*., 2000). Walker (1974) observed *R. evertsi* and *A. gemma* on zebras, and *R. praetextatus, R. appendiculatus, R. evertsi, R. simus*, *A. gemma*, and *D. rhinocerinus* on donkeys. However, Cumming (1998) reported *Hy. albiparmatum*, *Hy. impeltatum*, and *Hy. rufipes* on zebras and Hawkins *et al*. (2015) reported *Hy. truncatum* and *Hy. marginatum* on donkeys none of which were observed in this study. The geographical distributions of tick species are affected by the climate (Cumming, 1998). Additionally, places with high density of vertebrate and invertebrate host species have greater tick diversity compared to that of other areas (Turpie and Crowe, 1994; Cumming, 1998). The difference in tick species compared to past studies on zebras and donkeys might be due to the different localities and climates in which the zebras and donkeys were found.

This study also observed *Theileria equi* in zebras and donkeys, as well as *Anaplasma* cf. *platys* in donkeys. The prevalence of *T. equi* was high in zebras and donkeys. The previously reported prevalence of *T. equi* in zebra and Cape Mountain

zebra, 82% and 100%, respectively (Bhoora *et al*., 2010), and 100% in Grevy's zebra (Hawkins *et al*., 2015), were similar to that observed in the present study.

In donkeys, previous studies reported *T. equi* prevalence rates of 81.2% in Nuu division (Oduori *et al*., 2015), and 72% in Northern Kenya (Hawkins *et al*., 2015). In other countries, *T. equi* was observed in 41.2% of donkeys in Nigeria (Onyiche *et al*., 2019), 55.7% of donkeys in Ethiopia (Gizachew *et al*., 2013), and 26.6% of donkeys in Egypt (Mahmoud *et al*., 2016). Thus, the findings of the present study were similar to those previously reported (Oduori *et al*., 2015; Hawkins *et al*., 2015). Additionally, nine *T. equi* haplotypes were isolated from donkeys in this study. Zhao *et al*. (2020) examined 28 blood samples from donkeys/mules and reported five *T. equi* haplotypes. In Mexico, Romero-Salas *et al*. (2021) reported that 35 blood samples from donkeys from seven different locations showed only one *T. equi* haplotype. Likewise, Qablan *et al*. (2013) reported one *T. equi* haplotype among 67 blood samples from donkeys in Jordan. Therefore, the present study observed a larger variety of *T. equi* haplotypes compared to previous studies. This was probably because the donkeys observed in this study grazed in several places, while also grazing with other livestock and wild animals.

A partial 16S rRNA sequence similar to that of *Anaplasma* cf. *platys* was also found in donkeys in the present study. *Anaplasma* cf. *platys* is closely related to *A. platys* (Dahmani *et al*., 2019), which is normally detected in dogs and causes a zoonotic disease (Arraga-Alvarado *et al*., 2014). Previous studies also reported *A. platys* in cattle (Lorusso *et al*., 2016a), sheep (Dahmani *et al*., 2019), goats (Chochlakis *et al*., 2009), camels (Li *et al*., 2015; Lorusso *et al*., 2016b), red deer (Li *et al*., 2016), and buffalo (Machado *et al*., 2016). Dahmani *et al*., (2019) reported *Anaplasma* cf. *platys* prevalence rates of 19.8% in sheep, 27.7% in goats, and 22.6% in cattle. Other studies

reported *Anaplasma* cf. *platys* in cattle in Algeria (Dahmani *et al*., 2019) and Tunisia (Said *et al*., 2017), in sheep in South Africa (Berggoetz *et al*., 2014), and goats in China (Zhang *et al*., 2012). Dahmani *et al*. (2019) reported that this species is described as a neutrophil tropic *Anaplasma* spp. in ruminants. Further study is needed to confirm the presence of *Anaplasma* cf. *platys* in donkeys and their potential role in its transmission.

In this study, the overall prevalence of gastrointestinal parasites in zebras was 99%. Other studies in Kenya reported gastrointestinal parasite prevalence rates in zebras of 100% in Ol Pejeta (VanderWaal *et al*., 2014), 100% in Samburu County (Knafo, 2008), and 100% in Isiolo county (Wambwa *et al*., 2004). In other countries, the reported prevalence rates were 98% in Tanzania (Seeber *et al*., 2020), 98% in Namibia (Turner and Getz, 2010), 100% in South Africa (Krecek *et al*., 1987), and 54.7% in Nigeria (Atuman *et al*., 2019). Most previous studies reported gastrointestinal parasites in 90-100% of zebras. Therefore, the findings of the present study were consistent with previous reports. The present study identified the following 9 families/genera of gastrointestinal parasites from zebras: ciliates, *Anoplocephala* sp., *Parascaris* sp., strongyle, *Trichostrongylus* sp., *Dictyocaulus* sp., trematode, *Oxyuris* sp., and *Habronema* sp. Other studies in Kenya observed *Strongylus* spp., *Triodontophorus* spp., *Cyathostomum* spp., *Crossocephalus* spp., *Oxyuris* sp., *Habronema* sp., *Setaria equina*. *Parascaris equorum*, *Anoplocephala perfoliata*, *Strongyloides* sp., *Eimeria zuernii*, *Entamoeba* spp., trematode, and *Haemonchus* spp. in zebras (Wambwa *et al*., 2004; Knafo, 2008; VanderWaal *et al*., 2014). As *Haemonchus* spp. is a common gastrointestinal parasite in sheep and goats it was not detected in the present study as those animals were not included.

Setaria equina is the group of Onchoceridae whose intermediate host is *Aedes aegypti* and *Culex pipiens*. However, these intermediate hosts are probably not present in the geographic region in which this study took place. Therefore, *S. equina* was not detected. *Eimeria* spp. and *Entamoeba* spp. were also not observed, although oocysts were found. The morphology of the oocyst was too small to enable species identification by sugar flotation or modified sedimentation techniques. Thus, future studies should use other methods such as culture or molecular techniques.

The prevalence of strongyles in zebras in the present study was 99%. Wambwa *et al*. (2004) reported a 90% prevalence of strongyles in Burchell's zebra in Isiolo county. VanderWaal *et al*. (2014) also reported a 100% prevalence of strongyles among zebras in Ol Pejeta. Moreover, Knafo (2008) reported a 74.2% prevalence of strongyles among zebras in Samburu County. In other countries, the prevalence rates of strongyles among zebras were 24.5% in Nigeria (Atuman *et al*., 2019), >98% in Namibia (Turner and Getz, 2010), 98% in Tanzania (Seeber *et al*., 2020), and 100% in South Africa (Krecek *et al*., 1987). Except for Nigeria, most studies observed that >90% of zebras were infected with strongyles. Therefore, strongyles could be the main cause of the high prevalence of nematodes in zebras.

The second highest prevalence rate in zebras was observed for *Anoplocephala* spp. (27%). Other studies reported *Anoplocephala* spp. prevalence rates of 3.2 % (Knafo, 2008) and 80% (Wambwa *et al*., 2004) in Kenya. In Namibia, 7.6% of zebra were infected with *Anoplocephala* spp. (Atuman *et al*., 2019), and Scialdo *et al*. (1982) recovered *Anoplocephala* sp. in Burchell's zebra in South Africa. Therefore, this genus may be the most common gastrointestinal parasite in zebras.

Trichostrongylus spp. were detected in 16% of zebras in the present study. These

species were also observed in 22.6% of zebra in Samburu County (Wambwa *et al*., 2004). VanderWaal *et al*. (2014) also recovered *Trichostrongylus* spp. from 17% of zebras. Moreover, 44% of Burchell's zebras were infected with *Trichostrongylus* spp. in South Africa (Krecek *et al*., 1987). *Trichostrongylus* spp. is a globally distributed gastrointestinal parasite (Lichtenfels, 1975). However, some places in Kenya did not observe *Trichostrongylus* spp. in zebras (Wambwa *et al*., 2004). Unexpectedly, the prevalence of *Trichostrongylus* spp. was not high despite the global distribution of the genus. The reason for this anomaly could be that the sample size for this genus may have been too small to allow comparison to other gastrointestinal parasites of equids; thus, this genus may be easily overlooked (Pandey *et al*., 1992).

The prevalence of *Parascaris* sp. among zebras in this study was 6%. Another study observed *Parascaris* spp. in 25.8% of zebras (Knafo, 2008). Wambwa *et al*. (2004) reported a 30% prevalence of *Parascaris* spp. in Isiolo district. In Tanzania, the prevalence of Ascaridae, which is the same group as *Parascaris* spp., was 31% in zebra (Seeber *et al*., 2020). The prevalence of *Parascaris* spp. in the present study was lower than that reported previously. This difference could be attributed to differences in ecological and climatic environments across geographic areas. Knafo (2008) suggested that *Parascaris* spp. might be a more host-specific parasite compared to other parasites.

The prevalence of trematodes in this study was 2%. Another study discovered fluke eggs from 32.3% of zebras (Knafo, 2008). In Namibia, the prevalence of *Fasciola* sp. was 17% (Atuman *et al*., 2019). However, no other studies reported *Fasciola* spp. or other trematodes in zebras (Young *et al*., 1973; Wambwa *et al*., 2004; Turner and Getz, 2010; VanderWaal *et al*., 2014). Snails are the intermediate host of trematodes, and the greatest distribution of snails is around marshy or marginal shallow water areas (Phiri *et*

al., 2007; Singla *et al*., 2017). The prevalence of trematodes in this study was lower than those in other studies, likely because the environment was unsuitable.

Dictyocaulus arnfieldi is the primary lungworm affecting donkeys, horses, ponies, and zebras worldwide. *D. arnfieldi* is relatively common in donkeys (Matthews and Burden, 2013). In Grevy's zebra, the prevalence of *Dictyocaulus* sp. was 14.10% in Samburu County (Mwatenga, 2017). However, *Dictyocaulus* sp. was detected in only 3% of zebras in the present study. Other studies in Kenya and other countries did not observe *Dictyocaulus* sp. in zebras. Therefore, the genus could be considered an accidental occurrence in the present study.

Gasterophilus larvae are common parasites in the gastrointestinal tract of equines including horses, donkeys, and zebras. The present study observed *Gasterophilus* sp. in 1% of zebras. Wambwa *et al*. (2004) discovered *Gasterophilus* sp. from 100% of Burchell's zebras. However, few reports exit regarding the prevalence of *Gasterophilus* sp. in zebras. In this study area, the density of *Gasterophilus* fly could be lower than in other areas.

The present study observed *Oxyuris* sp. in 1% zebras. Wambwa *et al*. (2004) reported a 40% prevalence of *Oxyuris* sp. among Grevy's zebrawas 9.40% (Mwatenga, 2017). In South Africa, the prevalence of *Oxyuris equi* in Burchell's zebra was 84% (Krecek *et al*., 1987). The prevalence among zebras in the present study was lower than that in other studies (Krecek *et al*., 1987; Mwatenga, 2017). This low prevalence could be due to the relatively high temperatures in this study area, which desiccated the highly susceptible *Oxyuris* sp. eggs.

The prevalence of *Habronema* sp. in zebras in this study was 1%. However, Wambwa *et al*. (2004) observed *Habronema* sp. in 30% of Burchell's zebras in Isiolo

district. In South Africa, the prevalence of *Habronema* sp. in Burchell's zebra was 96% (Krecek *et al*., 1987). The intermediate hosts of *Habronema* sp. are *Muscae domestica* and *Stomoxys calcitrans*. The low numbers of *Habronema* sp. discovered in the present could be due to weather conditions not suitable for vectors during the study period. Moreover, *Habronema* sp. are found free in the stomach after death (Lyons *et al*., 1987). Therefore, most *Habronema* sp. might be associated with the mucosa; however, mucosal digestion was not performed in the present study.

In this study, the overall prevalence of gastrointestinal parasites in donkeys was 100%. Knafo (2008) and Mulwa *et al*. (2020) observed gastrointestinal parasites in 100% and 56% of donkeys, respectively. Regionally, the reported prevalence rates of gastrointestinal parasites in donkeys were 82.5% in Ethiopia (Belay *et al*., 2016), 27% in Nigeria (Ahmed *et al*., 2008), and 70% in Uganda (Saul et al., 1997). These differences are probably due to differences in geographical areas (Ibrahim *et al*., 2011; Raja *et al*., 2014) or diagnostic techniques (Mateus *et al*., 2014).

The present study detected eight families/genera of gastrointestinal parasites in donkeys: ciliates, *Anoplocephala* sp., *Parascaris* sp., strongyle, *Trichostrongylus* sp., *Dictyocaulus* sp., *Oxyuris* sp., *Habronema* spp., and *Strongyloides* sp. Other studies in Kenya observed *Strongylus* spp., *Trichostrongylus* sp., *Trichomonas* spp., *Parascaris* sp., *Strongyloides* sp., *Haemonchus* spp., *Oesophagostomum* spp. and trematode (Knafo, 2008; Mulwa *et al*., 2020).

The differences in species of gastrointestinal parasites among studies could be caused by differences in ecological and climate environments, sample sizes, and detection techniques (Robertson *et al*., 2000).

The prevalence of strongyle in donkeys in the present study was 95%. Mulwa *et*

al. (2020) observed strongyle in 44.7% of donkeys. In the Isiolo district, the prevalence of strongyle was 93.5% (Knafo, 2008). In Uganda and Nigeria, the prevalence of rates of strongyle were 70% (Saul et al., 1997) and 78.3% (Jajere *et al*., 2016), respectively. Other also studies reported high prevalence rates of strongyle (Wells *et al*., 1998; Ahmed *et al*., 2008; Burden *et al*., 2010; Chitra *et al*., 2011). Therefore, strongyle was likely to be the most dominant gastrointestinal parasite in donkeys compared to other parasites.

Trichostrongylus axei is a parasite of equids worldwide. This study observed *Trichostrongylus* sp. in 61% of donkeys. Another study in Kenya reported a prevalence rate of *Trichostrongylus* sp. of 35.5% (Knafo, 2008). However, Wambwa *et al*. (2004) and Mulwa *et al*. (2020) did not observe *Trichostrongylus* sp. in donkeys despite the similarity of these studies. In South Africa, Wells *et al*. (1998) reported a *Trichostrongylus* sp. prevalence of 2.8%. Getachew *et al*. (2010a) observed this genus in 91.3% of donkeys in Ethiopia. Ismail *et al*. (2016) discovered *Trichostrongylus axei* in 62% of donkeys in Sudan. Thus, the findings of the present study were consistent with those of Getachew *et al*. (2010a) and Ismail *et al*. (2016). The differences in the prevalence or absence could be attributed to different methods of diagnosis and species of animal involved.

The present study observed *Parascaris* sp. in 18% of donkeys. This result was broadly consistent with the findings reported by Knafo (2008) and Ismail *et al*. (2016), at 22.6% and 19.6%, respectively. However, this prevalence was lower than those reported by Ayele and Dinka (2010), Saul *et al*. (1997), and Jajere *et al*. (2016), at 32.5% in Ethiopia, 40% in Uganda, 40.3% in Nigeria, respectively. In contrast, the prevalence in the present study was higher than those reported by Wells *et al*. (1998)

(9.6% of donkeys in South Africa) and Mulwa *et al*. (2020) (5.3% of donkeys in Kenya). Getachew *et al*., (2010b) suggested that adult donkeys could harbor *Parascaris* sp. Therefore, *Parascaris* sp. could be a relatively common gastrointestinal parasite among donkeys in this study area.

Strongyloides sp. inhabits the small intestine and causes diarrhea and indigestion. Moreover, *Strongyloides* sp. infect foal equids through the mother's milk and are rarely been detected in adult equids. In this study, the prevalence of *Strongyloides* sp. was 13%, with most detected in juvenile donkeys. Knafo (2008) reported a prevalence of *Strongyloides* sp. of 41.9% in Kenya. Wells *et al*. (1998) reported a prevalence of *Strongyloides westeri* in South Africa of 24.8% based on faecal culture and 15.2% using the McMaster method. Moreover, Ibrahimet *et al*. (2011) reported a *Strongyloides westeri* prevalence in Ethiopia of 20% by Baermann technique. Saul *et al*. (1997) reported a *Strongyloides* sp. prevalence in Uganda of 30% by Baermann technique. The differences in prevalence rates could be attributed to the use of different diagnostic methods and the environment.

The prevalence of *Anoplocephala* sp. among the donkeys in this study was 2%, a rate similar to the rates reported by Mulwa *et al*. (2020), 2.5% *A. manga* and 10.3% *A. perfoliate* in Kenya. Saul *et al*. (1997) recovered *Anoplocephala* sp. in 10% of donkeys in Uganda. Ismail *et al*. (2016) also reported *Anoplocephala* sp. in Sudan. Other studies reporting gastrointestinal parasites from necropsy reported higher prevalence rates of *Anoplocephala* sp. compared to the present study, including 30% in Kiambu county (Lewa, 1999) and 60% in the US (Benton and Lyons, 1994). Therefore, this low prevalence may be attributed to the use of different diagnostic methods or the season for oribatid mite vectors.

Oxyuris equi is the common species infecting horses and other equids such as donkeys, mules, and zebras. In this study, *Oxyuris* sp. was detected in 2% of donkeys, consistent with the prevalence reported by Wells *et al*. (1998), Getachew *et al*. (2010b), and Mulwa *et al*. (2020) of 0.8% in South Africa, 2% in Ethiopia, and 1.1% in Kenya. In contrast, Ibrahim *et al*. (2011) and Jajere *et al*. (2016) reported prevalence rates of 31.8% in Ethiopia and 17% in Nigeria. Thus, the prevalence of *Oxyuris* sp. differed among areas, probably due to the impact of climatic conditions on the dynamics of egg excretion (Kuzmina *et al*., 2006). In addition, the prevalence rates varied among regions.

In Kenya, few studies have reported on gastrointestinal parasites of zebra and Grevy's zebra (Wambwa *et al*., 2004; Muoria *et al*., 2005; Knafo 2008; VanderWaal *et al*., 2014; Mwatenga 2017). Among these, only Knafo (2008) compared the prevalence of gastrointestinal parasites between zebras and donkeys, in which gastrointestinal parasites were found more commonly in zebras compared to donkeys. Ezenwa (2003) reported a broader variety of gastrointestinal parasites in the area with overlapping habitats of the host animals. Moreover, the host animal population density also affects parasite species richness (Nunn *et al*., 2003). The zebra is a free-ranging animal and most of the Maasai people who reside around this study area typically only possess one or two donkeys per household. This study also detected more ciliates, *Anoplocephala* sp., and strongyle in zebras compared to donkeys ($p<0.05$). In contrast, the prevalence of *Parascaris* sp. and *Trichostrongylus* sp. was higher in donkeys than in zebras ($p<0.05$). The difference in prevalence rates is affected by the host population density (Nunn *et al*., 2003). The results of this study suggest that some donkey owners dosed their donkeys with anthelmintic medication, which appears to be effective against some parasites. Thus, the difference in the prevalence of gastrointestinal parasites between

zebra and donkey is likely due to the host population density or anthelmintic drug uses.

5.2. Conclusion

1. More tick species were found on zebras compare to donkeys, with *Rhipicephalus pulchellus* ticks being the most abundant on both zebras and donkeys.
2. *Theileria equi* was the most common haemoparasite in both zebras and donkeys.
3. A partial 16S rRNA sequence similar to that of *Anaplasma* cf. *platys* was detected only in donkeys and could indicate a possible *Anaplasma* cf. *platys* infection in donkeys in Kenya.
4. Strongyle was the most common gastrointestinal parasite in zebras and donkeys with *Strongyloides* sp. being detected in donkeys. This suggests that donkeys may serve as potential reservoirs of zoonotic diseases.

5.3. Recommendation

1. The presence of a partial sequence similar to *Anaplasma* cf. *platys* identify from donkeys may indicate possible *Anaplasma* cf. *platys* infection in donkeys. Further molecular characterization involving whole mitochondrial sequencing should be conducted to further ascertain the exact identity of the 4 *Anaplasma* cf. *platys* isolate obtained from this study.
2. This study did not report any *Rhipicephalus sanguineus* and *R. annulatus* which are commonly known vectors of *Anaplasma* cf. *platys*. Therefore, *Anaplasma* cf. *platys* is confirm, then extensive surveillance of pathogens in ticks should be conducted in order to determine its possible vector.
3. This study used macroscopic examination to identify gastrointestinal parasites on faecal samples due to the limitation of this study, it was not possible to

identify the parasites to species level. Future studies should consider molecular techniques and faecal culture approaches.

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KENYA WILDLIFE SERVICE

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11 November 2019

Minori **KONO**
University of Nairobi
C/O P.O Box 30197-00100
NAIROBI
Email: minopyooon@gmail.com

Dear Minori

RESEARCH AFFILIATION WITH KENYA WILDLIFE SERVICE; NOVEMBER 2019-NOVEMBER 2020

We acknowledge receipt of your application requesting for research affiliation with Kenya Wildlife Service to conduct research on a project titled: ***Prevalence and Diversity of Parasite in Donkey Co-grazing with Zebra at Amboseli National Park and Adjacent Area.*** Following review of your project proposal as approved by the University of Nairobi, we note that the study will generate data and information that will be used in determining the prevalence and diversity of ticks, haemoparasites and gastrointestinal parasites in donkey co-grazing with zebra at the Amboseli National Park and its environment.

We are pleased to inform you that, your request has been approved and that you have been granted a Research Affiliation to conduct the study from November 2019- November 2020 upon payment to KWS, of Research Affiliation Fees of USD 120 (United States Dollars One hundred and twenty only) .

During the period of Affiliation, you will be required to abide by the set KWS Regulations and Guidelines regarding carrying out research in and outside Protected Areas. You will also be required to work closely with the KWS Veterinary Officer and the Senior Research Scientist in –charge of the Southern Conservation Area (SCA), to whom you will give the progress report on the study

You will submit a bound copy of your MSc thesis to the Director, Biodiversity, Research and Planning on completion of the study.

Yours

for: Kasiuki

PATRICK OMONDI, *PHD, OGW*
DIRECTOR, BIODIVERSITY, RESEARCH & PLANNING

Copy:
- Head, Veterinary Services (HVS)
- Senior Scientist, Southern Conservation Area (SRS-SCA)

Annex 1: The research affiliation from Kenya Wildlife Service