EFFECTS OF HABITAT OVERLAP ON HELMINTH TRANSMISSION BETWEEN SYMPATRIC BABOONS, VERVET MONKEYS AND UNGULATES IN AMBOSELI ECOSYSTEM, KENYA

A thesis submitted to the University of Nairobi in fulfillment of Doctor of Philosophy degree in Applied Veterinary Parasitology.

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DECLARATION

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

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This thesis is for the honour of two special people;

My wife, Alexia and son, Mich

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LIST OF ABBREVIATIONS

DNA	Deoxyribonucleic Acid
GPS	Global Positioning System
GSM	Global System for Mobile
ITS	Internal Transcribed Spacer
KM	Kilometers
MCP	Minimum Convex Polygon
OTU	Operational Taxonomic Unit
RNA	Ribonucleic Acid

ABSTRACT

Supervisors: Prof. Ndichu Maingi., Dr. Gerald Muchemi., Dr. Chege Nga'nga' and Prof. Elizabeth Archie.

In natural ecosystems, helminths and their diverse hosts co-exist as interacting ecological communities, however the factors that determine which helminths (s) infect which host (s) are not well understood. Habitat overlap is predicted to influence helminth transmission and infection patterns, including helminths co-occurrence across sympatric host taxa. The overall objective of this study was to determine the effects of habitat overlap between sympatric hosts on transmission of helminths in Amboseli ecosystem, Kenya. The focal animal was the baboon population, which has been the subject of studies for decades by the Amboseli Baboon Research Project, while the sympatric animals included Vervet monkeys, cattle, sheep, goats (domestic ungulates) and wildebeests, impala, Grant's gazelles and Thomson's gazelles (wild ungulates). The Amboseli baboon population is structured in to six social groups, which are well known in terms of their ranges and numbers.

The first specific objective of this study was to determine the degree of habitat overlap among the social groups of baboons and between baboons and the other sympatric host species. Key individuals of a baboon social group were tracked daily for 7days and (Global positioning system) GPS coordinates of their point locations uploaded in BIOTAS software to generate home ranges by Minimum Convex Polygons (MCP) method. The home range map, size (km^2) and degree of overlap between baboon groups were calculated using the software. The degree of overlap between baboon groups and other sympatric hosts was determined through frequency sightings of the sympatric hosts

and their dung pile counts in the home range of each baboon group. Results revealed that the home ranges of each baboon group at 100% MCP overlapped with the ranges of at least four other baboon groups, while home ranges at 50% MCP overlapped with at least one other baboon group home range. Degree of habitat overlap between baboon and sympatric hosts based on mean dung pile counts (94.2) was higher at 100% MCP compared to degree of overlap (25.6) at 50% MCP of baboon habitat. In addition the degree of habitat overlap between baboon and sympatric hosts based on mean frequency sightings was higher (8.8) at 100% MCP compared to degree of overlap (4.5) at 50% MCP of baboon habitat. These results indicated that the degree of overlap across baboon home ranges and between baboon and sympatric hosts varied according to the proportion of habitat used by baboons.

The second objective was to determine helminth prevalence, abundance and species richness in all the nine sympatric hosts. Sedimentation and floatation techniques were used to assess 1138 formalin-fixed faecal samples collected in the dry and wet season. A total of 16 types of helminth eggs were identified by both sedimentation and floatation fecal assessment methods. Out of these, eight were nematodes, which included Strongylids, *Enterobius* sp., *Strongyloides* spp., *Primasubulura* sp., *Trichuris* spp., *Streptopharagus* sp., unidentified species of *Spirurina,* and unidentified species of Spirurids. The cestodes included *Moniezia expansa* and *Moniezia benedini*. Trematodes included *Paramphistomum* sp., *Fasciola gigantica* and *Fasciola hepatica*. Differences in helminth prevalence were recorded across all host species (χ^2 = 200.37, df = 8, p = 0.0001) and between seasons (χ^2 = 23.87, df = 1, p = 0.0001), using the sedimentation method. Moreover, prevalence of helminths significantly differed across baboon social

groups (χ^2 = 22.43, df = 5, p = 0.0001) as well as between seasons with higher prevalence in the dry (χ^2 = 13.56, df = 1, p = 0.019) compared to the wet season (χ^2 = 18.26, df = 1, p = 0.003). The floatation method also revealed differences in prevalence of helminths across host taxa (χ^2 = 54.505, df = 8, p<0.0001). Prevalence of helminths across baboon groups differed significantly (χ^2 = 27.754, df = 5, p<0.0001) but not between seasons (χ^2 = 1.680, df = 1, p = 0.195). Helminth prevalence obtained by floatation method across all the nine sympatric host species was significantly higher (χ^2 = 157.472, df = 1, p<0.001) than when determined by sedimentation method. In contrast, helminth species richness was significantly higher when determined by sedimentation method (χ^2 = 132.703, df = 5, p<0.001) than when floatation method was used. Concordance between floatation and sedimentation methods was low (0.101) according to Cohens' kappa statistic. It was found that mean abundance of helminths varied across host taxa and across baboon social groups. Helminth species richness across the host species community ranged from two to eight, with mean species richness of 5.1 ± 1.9. These results indicate that in a sympatric host community, prevalence, abundance and species richness of helminths was highly variable across host taxa. Similar infection patterns were observed between social groups irrespective of their very close proximity of spatial overlap. Seasonality strongly influenced patterns of helminth infection within and across host species. It is likely that the factors that determine intergroup variation in helminth infection are multiple and includes the demographic structures of social groups such as age and sex and habitat heterogeneities.

The third objective was to test the influence of habitat overlap among baboon groups and between baboons and sympatric host species in determining helminth infection patterns.

Since the degree of home range overlap between baboon home ranges did not vary at 100% MCP, only values at 50% MCP were tested to determine influence of degree of habitat overlap on helminth prevalence, abundance and species richness. Further, dung pile count and frequency of sighting alternative hosts in baboon home ranges were used as indices of degree of overlap. Specifically, relationships were tested between dung pile counts, mean frequency of animal sightings, Shannon-Wiener diversity index, host species diversity and both helminths prevalence and abundance in baboons. The results indicated a lack of statistical association between degree of habitat overlap across baboon groups and their helminths prevalence, abundance and species richness ($p > 0.05$). These findings indicate that the degree of habitat overlap between social groups does not influence helminths prevalence, abundance and species richness.

Statistical analysis showed that at 50% MCP of baboon home ranges, the degree of overlap (based on dung pile counts) between baboon and sympatric hosts did not significantly influence mean helminth prevalence ($r^2 = 0.441$, $t = -1.777$, $p = 0.150$) and mean helminth abundance ($r^2 = 0.222$, $t = -1.068$, $p = 0.3458$). Results also indicated that the degree of habitat overlap between baboon and sympatric hosts indicated by Shannon-Wiener diversity index, host diversity and mean frequency of sightings, did not statistically influence helminth prevalence and abundance. However, host diversity ($r^2 = 0.665$, $F_{(1,4)} = 7.594$, $p = 0.05$) and Shannon-Wiener diversity index ($r^2 = 0.727$, $F_{(1,4)} = 10.64$, $p = 0.031$) significantly influenced helminths species richness in baboon groups. Both indices showed that the degree of habitat overlap between baboons and other sympatric host species did not significantly influence helminths prevalence and abundance in baboons. However, increased habitat overlap with more diverse

communities of sympatric hosts showed a declining trend in helminths species richness in baboons.

The fourth objective was to genetically determine the species of nematodes shared among sympatric baboons, vervet monkeys and ungulates in Amboseli ecosystem. A total of 977 DNA samples were extracted from larvae cultured from faecal material collected from all the sympatric hosts during the dry and wet seasons. The DNA was amplified by both mitochondrial and internal transcribed spacers of ribosomal genes followed by sequencing. A total of 67 sequences were used for identification of the nematodes and phylogenetic reconstruction. *Strongyloides stercoralis, Strongyloides fuelleborni, Trichostrongylus colubriformis,* and *Oesophagostomum bifurcum* were identified from baboons. *Cooperia oncophora* was identified from cattle, *Haemonchus contortus* from goats and *Teladosargia circumcincta* from Grant's gazelles. In addition, a nematode that displayed viviparity and identified to be a member of the sub-family *Cyathostominae* was shared in the six sympatric hosts; baboons, vervet monkeys, Thomson's gazelle, impalas, goats and cattle. Results indicated that habitat overlap facilitated Cyathostominae sharing across hosts, irrespective of their evolutionary relatedness, which may imply a host shift. Specifically, it was most likely that the viviparous nematode underwent a shift to colonize a new host range that includes unusual hosts for members of the sub-family *Cyathostominae*. Phylogenetic analysis of *Strongyloides fuelleborni* demonstrated geographical structuring rather than host structuring; specifically, the species in Kenya were genetically distinct from those previously found in Tanzania, Gabon and Japan. In addition, phylogenetic analysis revealed two genetic populations of *S. stercoralis* in baboons, which had different evolutionary trajectories. The baboon population harboured

helminth species of zoonotic potential (*S. fuelleborni, S. stercoralis, O. bifurcum, T. colubrifomis*), which is a risk to the pastoral community in Amboseli ecosystem.

Overall, the results from this study demonstrate that habitat range of baboon social groups exhibited variable overlap with other baboon groups, however overlap did not influence helminth infection patterns across social groups. Further, the overlap between baboons and other sympatric hosts was variable, but the degree of overlap did not significantly influence helminth prevalence and abundance, except helminths species richness across baboon groups. This study also found out that in sympatry, multiple species of helminths, *Cyathostominae, Trichuris* spp., *Moniezia benedini, Moniezia expansa, Primasubulura* sp., *Enterobius* sp., and Spirurina, were commonly shared but at different levels across host taxa, with some hosts harboring more helminths richness than others. Specifically, baboon harboured more helminths species than any host in the community. This is the first study in Kenya to determine helminth infection pattern in a multi-host community involving both wildlife and livestock that belong to multiple phylogenies. Presence of *Oesophagostomum bifurcum, Enterobius* sp., *Strongyloides* sp., *Trichostrongylus colubriformis* in the Amboseli animal community is of public health interest because of their zoonotic potential, hence this study recommends a study to determine their prevalence in the human community in the region.

CHAPTER ONE: GENERAL INTRODUCTION

1.1 Introduction

This study is in the field of disease ecology, which is the ecological study of host-pathogen interactions within the context of their environment and evolution (Kilpatrick and Altizer, 2010). It is therefore important to define some ecological terms in the context of this study because although widely used, they tend to be inconsistent, imprecise and ambiguous. The study was carried out in Amboseli ecosystem in Kenya, where ecosystem refers to any unit that includes all of the organisms in a given area interacting with the physical environment so that the flow of energy leads to clearly defined trophic structure, biotic diversity, and material cycles (Odum, 1971).

The term habitat is used here to refer to the area that has physical and biological resources as well as conditions that produce occupancy – including survival and reproduction – for a given organism (Hall *et al*., 1997). Habitats may be used for foraging, cover, nesting, escape or other life history traits (Hall *et al*., 1997). Habitats are thus heterogeneous in terms of resource availability and abundance both spatially and at temporal scales. As such, animals have to select which habitat they would use at different scales of the environment (Krausman, 1999).

Habitat selection entails a conscious behavioral process by which an animal searches for features within an environment that are directly and indirectly associated with resources it would need to reproduce, survive and persist (Krausman, 1999). According to Hilden (1965), habitat selection is governed by proximate and ultimate factors. The suitability of a site, which includes forage composition and source of water, serve as cues for selecting

sites. On the other hand, the ultimate factor that influences an animal to select a site is that which will enable it to achieve reproductive success and survival.

Since resource needs and habitat preference of diverse species tend to converge, it is common in nature to find multiple species sharing a habitat. Therefore, in such habitats, it is normal to find two species or populations that physically encounter one another with high frequency, an interaction termed as sympatry (Mallet *et al*., 2009). Fitzpatrick *et al*., (2008) explains further that when the range of one species is included in the range of the other such that the union of the two ranges is equal to the larger of the two, then the organisms occur in complete sympatry. This is in contrast to partial sympatry in which only a portion of the area of the two geographical ranges overlap but also areas where only one of the two organisms occupy.

Such sympatric interaction was specifically selected to include populations of baboon, vervet monkey and ungulates in Amboseli ecosystem. Baboon and vervet monkey are both old world non-human primates indigenous to Kenya. Baboons are the largest of all the monkeys. Ungulate is a general term that includes all animals with hooves.

The term parasite describes organisms that live in or on and obtain resources from a host, usually to the host's detriment (Kilpatrick and Altizer, 2010). Parasites are generally grouped into microparasites and macroparasites to reflect differences in their population biology (Anderson and May 1979). Microparasites (which include viruses, bacteria, fungi and protozoa) are microscopic organisms that reproduce and multiply inside their hosts on rapid timescales (Kilpatrick and Altizer, 2010). In contrast, macroparasites (mostly parasitic worms called helminths and parasitic arthropods such as lice) are larger, longer-

lived, and hardly complete their life cycle within a single host (Kilpatrick and Altizer, 2010). Instead, adult macroparasites usually shed infective life stages such as eggs or larvae into the environment, and these may or may not infect the same host that the adult macroparasites live in (Kilpatrick and Altizer, 2010).

This study focused on helminths, a general term that refers to parasitic invertebrates characterized by elongate, flat or round bodies (Castro, 1996). Helminths infect nearly all mammalian organisms when their eggs or larval stages are ingested or when larvae actively penetrate a definitive host in which they mature to adult stage.

Parasites, which include helminths cause weaknesses that negatively impact fitness, fecundity and population dynamics of hosts to the extent they threaten host survival and speed up extinction risks (Daszak *et al*., 2000; Ferber, 2000; Deem *et al*., 2001; Nunn and Altizer, 2006). It is therefore imperative to understand factors that drive transmission rates and shape infection patterns in ecological communities. Understanding which factors drive transmission rate and shape patterns of infection especially in the context of ecological structure can help in prediction and mitigation of emerging infectious diseases. Most studies on patterns of parasite infection have focused on factors that are intrinsic to single host species, such as habitat structure and ecology (Chapman *et al*., 2006a; Chapman *et al*., 2006b), host behaviour (Cote and Poulin, 1995), host phenotypics (Ezenwa *et al*., 2006; Ezenwa and Jolles, 2008) and host genetics (Luikart *et al*., 2008; Rijks *et al*., 2008; Kloch *et al*., 2010). In contrast, nature presents a complex system where multi-host communities co-occur (habitat overlap) and interact with numerous pathogenic and non-pathogenic parasites (Belden and Harris, 2007). Co-occurrence of hosts (habitat overlap) is one of the factors that enhances contact rates between hosts and

parasites, hence a critical driver of disease transmission or spread (Hudson *et al*., 2002). Thus, habitat overlap is likely to influence infection patterns and composition of parasite communities as it brings together hosts and parasites of diverse evolutionary lineage.

The few studies that have explored how host habitat overlap influences infection patterns and disease within ecological communities (Woolhouse *et al*., 2001; Ezenwa, 2003; Johnson *et al*., 2013) suggest that for fecally-transmitted nematodes, which accumulate in pasture and soil, habitat overlap likely enhances opportunities for their transmission. This implies that for these parasites, physical contact between hosts is not necessary. Rather, spatial overlap between sympatric host species is probably sufficient for cross-species transmission (Ezenwa, 2003; Ocaido *et al*., 2004; Ekanayake *et al*., 2006; Jones *et al*., 2008; Howells *et al*., 2011; Archie and Ezenwa, 2011). Further, habitat overlap seems to nurture parasite adaptive traits which facilitate host shifts, defined as movement of a parasite from its traditional host to a new one (Antonovics *et al*., 2002). It is predicted that in areas of great biodiversity, pathogens are likely to undergo host shift, hence hot spots for high incidence of emerging and re-emerging diseases (Jones *et al*., 2008). This means that as habitat ranges constrict leading to greater overlap, parasite host ranges are likely to expand.

The diversity and sympatry of hosts in Amboseli ecosystem presented a suitable natural model to investigate how such a community affects the infection pattern of gastrointestinal helminths. Specifically, the aim of this study was to determine how the overlap of baboon habitat with alternative hosts (Vervet monkey and ungulates) in the Amboseli ecosystem might influence patterns of gastrointestinal helminth infections in baboon populations. Savanna baboons share habitat and similar diets with other non-

human primates and several vertebrate grazers. Gastrointestinal parasites are common in all these species and because many of these parasites infect multiple host species, interactions among host species will most likely influence dynamics of parasite transmission in this community.

1.2. Research Questions

This study addressed the following research questions; (1). Do baboon social groups vary in their habitat overlap with each group and alaternative hosts? (2). Do differences in the degree to which baboon groups habitats overlap with each group and alternative hosts explain differences in patterns of helminths infections and helminth communities across baboon groups? To address these questions, studies on habitat ranges and overlaps were carried out and both coproscopic and molecular techniques applied to identify the helminth communities.

1.3 Objectives

1.3.1 Overall objective

To determine effects of habitat overlap on helminth infection patterns (prevalence, abundance, species richness) and to genetically determine which species of nematodes are shared among sympatric baboons, vervet monkeys and ungulates in Amboseli ecosystem in Kenya.

1.3.2 Specific objectives

i. To determine the degree of habitat overlap among baboon social groups and between baboons and alternative host species in Amboseli ecosystem.

ii. To determine helminth prevalence, abundance and species richness in the sympatric hosts in Amboseli ecosystem.

iii. To determine the influence of habitat overlap among baboon social groups and between baboons and alternative host species on helminth infection patterns.

iv. To genetically determine the species of nematodes shared among sympatric baboon, vervet monkey and ungulates in Amboseli ecosystem.

1.4 Justification

In nature, wild animals and livestock co-occur and share diverse landscape resources. Such interactions create risk for pathogen sharing since many diseases are shared by wildlife and livestock, though general perception assumes a unilateral transmission pathway from wild animal hosts to domestic animal hosts. Helminths play important ecological functions in the population dynamics of wild animals, whereas in livestock production, they cause huge economic losses (Tisdell *et al*., 1999; Perry and Randolph, 1999). As such understanding ecological factors that regulate their transmission could contribute to their management, control and prevention. Most of the information about parasite-host interaction is usually based on systems that involve a single host and a single parasite. Since the natural or normal situation is that parasite-host interaction is much more intricate and involves assemblages of hosts and parasites, it is imperative to understand which factors drive and shape transmission patterns within an ecological community.

CHAPTER TWO: LITERATURE REVIEW

2.1 Host ecology of study animals

2.1.1 Baboons

Baboons are semi-terrestrial cercopithecine old world monkeys in the genus *Papio,* which occupy vast ecosystems across the continent of Africa (Altmann and Altmann, 1970; Kingdon, 1997). There are five species of baboons which include the Olive baboon (*P. anubis*) and Yellow baboon (*P cynocephalus*) occurring in the North, East and Central Africa; Chacma baboon (*P. ursinus*) in South Africa; the western/red/guinea baboon (*P. papio*) in western Africa and the Hamadryas baboon (*P. hamadryas*) in the horn of Africa extending to parts of Arabia. The Hamadryas baboons (*Papio hamadryas*) exhibit a social system that is different from the rest of the baboon species across Africa (Stammbach, 1987; Alberts *et al*., 2005). As such, the other four species of baboons, except the hamadryas, are generally referred to as the 'savanna' baboons (Alberts *et al*., 2005). According to Kamilar *et al*., (2006), the savanna baboons occupy significantly distinct environments, yet display a difference in their diet, activity budget, and social organization.

In Kenya, there are two species of baboons, Olive (Figure 2.1A) and Yellow baboon (Figure 2.1B), which hybridize in locations where their ranges overlap, especially in the southern parts of Kenya and northern parts of Tanzania. For instance, the present study focused on the baboon population in Amboseli ecosystem, where both species overlap and hybridize to produce a sub-species, *P. cynocephalus ibeanus* (Alberts and Altmann, 2001).

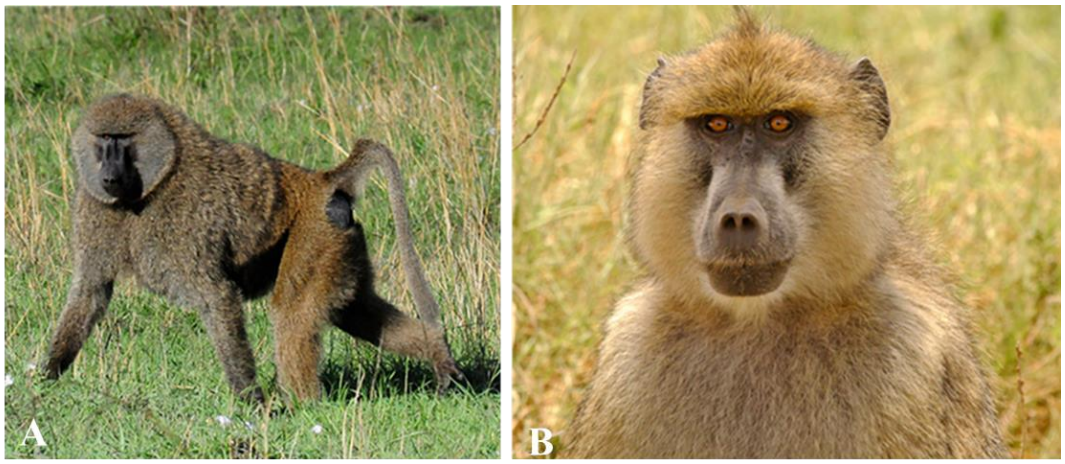


Figure 2.1 (A) Olive baboon and (B) Yellow baboon. Images courtesy of benneus.com and Paul mannix.

Baboons are 'eclectic' omnivores with an extensive diet range but highly selective in their forage pattern (Muruthi *et al*., 1991). Vegetation constitutes the main diet, which is supplemented selectively with invertebrates (insectivory) and opportunistically with vertebrates (Alberts *et al*., 2005). Baboons spend most of the day time on the ground foraging and socializing, which begins at around 7am (Wahungu *et al*., 2001). The animals return to specific trees, used as sleeping groves at dusk, around 5.30-6.45pm (Wahungu *et al*., 2001; Altmann and Altmann, 1970). They tend to rotationally change their sleeping groves, which have been hypothesized as anti-predation and anti-parasitic strategies (Hausfater, 1982).

The baboon society is organized in social structures referred to as troops, which is a multi-male and multi-female group composed of 15 – 150 individuals (Estes, 1991; Ray and Sapolsky, 1992). This means that a single baboon population consists of multiple social groups with varying home ranges that may or may not overlap (Altmann and

Altmann, 1970). Individual adult males may disperse in search of female mates in other social groups, but generally, they remain resident in the group year round (Alberts and Altmann, 1995). Females remain in their natal groups for their entire lives (Barton *et al*, 1996). Mating and giving birth in baboon occurs at any time of the year implying they do not have seasonal patterns of mating or birthing (Bercovitch and Harding, 1993; Bentley-Condit and Smith, 1999). Baboons are sexually promiscuous animals since both males and females have multiple mates (Cawthon, 2006).

The baboon society is hierarchical with dominance being ranked and among males rank is contested, defended and reinforced through both agonistic and friendlier interactions (Smuts, 1985; Cawthon, 2006). In contrast, dominance hierarchy among females is separate from that of males within the group (Packer and Pusey, 1979) and rank is inherited by linear matrilineal hierarchy (Smuts, 1985). This means that rank is passed down through the mother, which implies that daughters rank just below their mothers, while related females are ranked lower or higher than other females of matrilineal kin (Cawthon, 2006). Although home ranges of each social unit is defined and defended, sometimes they overlap, with the larger group displacing the smaller group (Smuts, 1985). Inter-group encounters are characterized by males being defensive of their females in the group (Parker, 1979).

The ecological and behavioural aspects of the baboon population in Amboseli ecosystem have been continually studied since 1971 by the Amboseli Baboon Research Project. As such social groups are known and named as well as individuals in each of the social groups.

2.1.2 Vervet Monkeys

Vervet monkeys are medium-sized cercopithecine old world monkeys (Figure 2.2) that are indigenous to Africa where they occur in 39 countries (Cawthon, 2006). There are six species of vervet monkeys in the genus *Chlorocebus* (Groves, 2001; Grubb, 2003). Of all the African monkeys, vervets are the most wide spread, occupying almost the entire sub-saharan Africa but strikingly absent in the Congo forest basin (Wolfheim, 1993). All the species of vervets are sexually dimorphic, with males having distinct blue coloration in the scrotal area (Cawthon, 2006). They are semi-arboreal and semi terrestrial, giving them advantage to exploit wide range of habitats and ecosystems (Fedigan and Fedigan, 1988). Although species are geographically separated, hybridization occurs in areas of overlap (Groves, 2001). The most widespread species of vervet found in Kenya is the *Ch. pygerythrus*, whose geographical range extends from Ethiopia, Sudan, Southern Kenya, Tanzania and eastern Uganda. The other species is *Ch. tantalus*, whose range in Kenya is northwestern parts around Lake Turkana (Cawthon, 2006). The other species of vervet monkeys are *Ch. cynosuros, Ch. djamdjamensis, Ch. aethiops* and *Ch. Sabaeus* (Cawthon, 2006).

Vervets feed on a wide range of diets, with preference on fruits and flowers, but they also feed on arthropods, lizards, rodents, birds and other vertebrate prey (Cawthon, 2006). Vervets share much of their ranges with baboons, but strikingly different is their adaptation to savanna habitats (Isbell, 1990) as well as strong seasonality in their mating and birthing. The vervet monkey population in Amboseli National Park has peak birthing from October to January (Cheney *et al*., 1988). These monkeys live in social groups whose compositions vary in size, age and sex (Struhsaker, 1967). In Amboseli, group size

may range from seven to 53 individuals with a mean group size of 24.1 (Struhsaker, 1967). Changes in group size are due to mortality, natality, emigration and immigration (Struhsaker, 1967). Social groups defend home ranges from intruding groups or individuals from other social groups (Struhsaker, 1967).

Figure 2.2. Male vervet monkey (left) and female with young one (right). Images courtesy of Ano and Louise Meintjes

2.1.3 Impala

Impala is a medium-sized African antelope in the genus *Aepyceros* and in the family *Bovidae*. There are two species, the common impala (*A. melampus*) and the black faced impala (*A. petersi*). Impala is widespread in many countries in the sub-saharan Africa. The species is sexually dimorphic, with males being larger bodied compared to females and bear long horns (Figure 2.3A), while females lack horns (Estes, 2004). The species occupies savanna grassland and open woodlands as it is a mixed feeder oscillating

between grazing and browsing depending on seasonal and habitat pasture richness (Wronski, 2002). The social organization of impala is structured into three units, which is however only conspicuous during wet season; the bachelor herd (consists of males only), female herds and the territorial males. The bachelor herd comprises of upto 30 individuals of mixed young males and non-territorial adult males (Murray, 1981). The female herd is conspicuously large comprising of 15-100 individuals, which includes breeding females and their young (Murray, 1981; Murray, 1982). The social ecology of impala is strongly influenced by season; home range size, mating, birthing, territoriality (Schenkel, 1966; Murray, 1981; Murray, 1982). Impala herds prefer locations near water to avoid long distance migration and tend to occupy home range size of 0.5 - 1.1 km^2. Leadership is not distinct, even among the female herd (Murray, 1981). Dominance among males is obtained through fights between rutting males. Territorial males spend more energy searching for estrous females than it uses for feeding and grooming during mating season (Mooring and Hart, 1995). Males demarcate their territory using faeces and dung heaped as middens and defends the territory against intruding males (Schenkel, 1966).

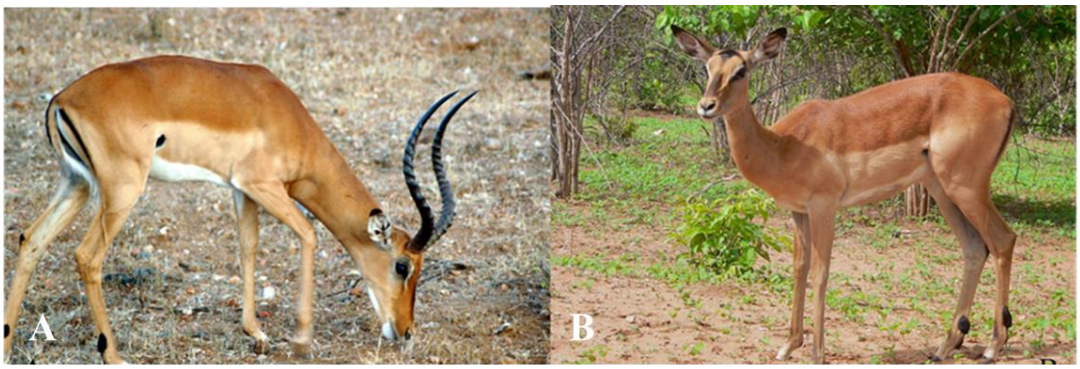


Figure 2.3: (A) Male and (B) female Impala. Image courtesy of galleryhip.com and sodahead.com.

2.1.4 Grant's Gazelles

Grant's gazelle is one of the many African antelope species in the genus *Nanger* and family *Bovidae* (Figure 2.4). This gazelle occupies savanna open grassland including shrub areas extending to semi-arid areas (Arctander *et al*., 1996) in Kenya, Sudan, Ethiopia and Tanzania (Nowak, 1991). They generally prefer dry plains in the dry season and retreat into the woodlands in the wet season. This species exhibits both migratory and territorial traits (Estes, 1991). The Grant's gazelles are mixed feeders, utilizing both browse and grass (Oindo, 2002) but are interdependent of water as they derive sufficient water from plants (Walther, 1972).

Figure 2.4: Grant's gazelles. Image courtesy of the author © 2015

The social structure and behavior: group size, mating, birthing are all influenced by ecological factors (Estes, 1991; Gerard *et al*., 2002). Stuart and Stuart, (1997) describes several social units in the Grant's gazelle population, which include bachelor unit, female unit and the dominant male unit. The female unit comprise of breeding individuals and their young ones whereas bachelor unit comprise of non-territorial and sub-adult males

(Walther, 1972). The dominant males demarcate their territories with urine and faecal middens and defend their territories from other male intruders (Walther, 1991).

2.1.5 Thomson's Gazelles

Thomson's gazelle is the most known little African antelope, which is in the genus *Eudorcas* and family *Bovidae* (Figure 2.5). This gazelle is wide spread in Kenya and Tanzania, occupying open savanna grassland as it is a mixed feeder (Estes, 1991; Kingdon, 1997). They are considered more drought resistant than most of the ungulates (Kingdon, 1982). They prefer foraging on short grass especially in areas where larger herbivores have grazed or burnt areas with re-growing grass (Kingdon, 1982). This means they are closely associated with large ungulates like cattle, wildebeest and zebra (Kingdon, 1982). Ecological factors influence social behavior, whereby in the wet season, perhaps due to the abundance of food, adult males establish breeding territories, which they defend from intruding males (Walther, 1977). Members of the bachelor group are restricted from entering the defended territories (Jarman, 1974). Females establish their group which comprises breeding individuals that criss-cross the male territories (Jarman, 1974). Territorial males usually attempt to herd the female group in order to restrict them in its territory however most often it may only retain one (Jarman, 1974; Estes, 1991). The bachelor herd gain dominance by fights, while territorial males tend to perform ritual behaviours of mock fights (Estes, 1991). Territorial boundaries are demarcated by secretions smeared on grass stems from preorbital glands (Estes, 1991). Territorial males do not enter territories of others even if a female escapes into another male territory. Territories are relinquished when populations make seasonal habitat shifts (Kingdon, 1982).

Figure 2.5: Female Thomson's gazelle with a newborn (left), male Thomson's gazelle (right). Image courtesy of Burrard Lucas.com.

2.1.6 Wildebeests

Wildebeest, commonly known as 'gnu', is in the genus *Connochaetes* in the family *Bovidae* (Figure 2.6). There are two species of wildebeest, the blue (*C. taurinus*) and black (*C.gnou*). The two species are differentiated morphologically by their body and tail colors and horn orientation. Blue wildebeest tend to be dark with grey stripes with bluish sheen, black tail and horns that are curved outwards before curving up. They are generally larger than the black wildebeest. In contrast, the black wildebeest has brown sheen, white tail, and the horns are curved downwards before curving up (Estes, 2014). The black wildebeest is restricted to the southern tip of Africa, while the blue wildebeest is common in eastern and southern Africa ranges, which includes Kenya, Tanzania extending to Swaziland. Kenya has resident sub-populations in Amboseli National Park, Naivasha ranches and Maasai Mara National Reserve. Wildebeest prefers open plains of grassland, including floodplain grassland and open bush savanna (Estes, 1991) since greater proportion of their diet is grass. Wildebeest are highly gregarious and socially

advanced ungulates. Wildebeest exhibit both sedentary and migratory behaviors; some populations have regular home ranges while others undergo annual long distance migration (Estes, 2014). The resident populations occupy their ranges through the year, dispersed in single territorial and small segregated herds (Estes, 1991). The social structure includes three social units: territorial males, bachelor herds and breeding female herds. These structures are more pronounced in the black than in the blue wildebeests (Estes, 2014). The female herds consist of breeding females and their young ones (Estes, 1991). Territorial males demarcate their boundaries with urine and faecal middens and secreted scents (Estes, 1991). The bachelor herds consists of a loosely associated group of yearlings, sub-adult and adult males (Estes, 1991).

Figure 2.6: Population of blue wildebeest. Image courtesy of Zicasso.com

2.1.7 Cattle

Cattle, Family *Bovidae* and genus *Bos,* are the most common large livestock reared across the globe. Cattle are mainly grazers that consume a wide variety of vegetation types. They are managed under different husbandry systems ranging from traditional

free-ranging to the more intensive systems of zero grazing. Free range system of cattle husbandry includes pastoralism, which is common among some communities in Africa. Since pastoralists inhabit arid and semi-arid regions across Africa they are always in constant movement in search for available or quality pasture and water for their livestock across vast ecosystems. In Kenya, Maasai community is one of the pastoralist communities whose region tranverse the Amboseli ecosystem. The Maasai community keeps mixed breeds of cattle (Figure 2.7) comprising of the small East African shorthorn zebu, Boran and Sahiwal (Kategile and Mumbi, 1992; Nkedianye *et al*., 2011).

Figure 2.7: Mixed cattle breeds kept by the Maasai community in Kenya. Image courtesy of woodycampbell.com.

2.1.8: Goats

Goats are medium sized ruminant livestock in the family *Bovidae* that occur worldwide and are mainly kept for meat and milk. They are excellent browsers that utilize wide range of herbage including fallen leaves and pods of dicots. Goats of the pastoralists also range extensively as the cattle in search of pasture and water. The Maasai community in

Amboseli region mainly keeps mixed breeds of goats (Figure 2.8) that includes the Galla and the small East African goat (Kategile and Mubi, 1992; Nkedianye *et al*., 2011).

Figure 2.8: Mixed goat breeds kept by the Maasai Community in Kenya.

2.1.9: Sheep

Sheep is a medium sized livestock in the family *Bovidae* that is found worldwide under variable systems of husbandry. Among the pastoralists, sheep is of great socio-economic importance that is reared under extensive ranging system across vast ecosystems. Sheep are grazers utilizing plants that are very close to the ground. Pastoralists prefer drought and disease tolerant breeds such as the Red Maasai sheep, East African fat-tailed sheep, the Black-Headed Persian and the Dorper (Figure 2.9). In Amboseli region, the breeds kept are the Maasai red sheep and the black-head Somali (Nkedianye *et al*., 2011).

Figure 2.9: Breeds of sheep reared by the Maasai community in Kenya. (A) Red Maasai sheep; (B) Dorper sheep; (C&D) East African fat-tailed female and male sheep.

2.2 Helminth infection in hosts

2.2.1: Helminths in baboon

Numerous helminth species have been encountered in baboons of which nematodes account for the largest diversity with the common species being in the genera *Oesophagostomum, Trichostrongylus, Trichuris, Strongyloides, Ternidens, Abbreviata, Molineus, Streptopharagus,* and *Physaloptera* (Muller-Graf *et al*., 1996; Munene *et al*., 1998; Hahn *et al*., 2003; Legesse and Erko, 2004; Gillespie *et al*., 2004; Kooriyama *et al*., 2012).

Among trematodes, *Schistosoma mansoni* is the most common in baboons of Africa and of public health importance because it is zoonotic (Munene *et al*., 1988; Murray *et al*., 2000; Erko *et al*., 2001; Legesse and Erko, 2004). The trematode has been reported in

several baboon populations of the Ethiopian Rift Valley (Erko *et al*., 2001; Legesse and Erko, 2004). In Kenya, prevalence of *S. mansoni* in wild caught baboons was found to be 4.3% (Munene *et al*., 1998).

Presence of cestodes in baboons is rare across Africa, with few incidences of *Bertiella studeri* mentioned in East Africa and Southern Africa (Nelson, 1965; Goldsmid, 1974). Kooriyama *et al*., (2012) found 1% prevalence of *Bertiella sp.* in baboons at Mahale Mountain National Park, Tanzania while Appleton and Brain, (1995) found 9.6% in a Mountain baboon population in South Africa.

Several aspects of helminth infection (prevalence, infection intensity, parasite diversity, transmission patterns) in baboons have been subject of many empirical studies (Munene *et al*., 1998; Legesse and Erko, 2004; Bezjian *et al*., 2008; Howells *et al*., 2011), which have demonstrated that the epidemiology of helminth infections vary among populations and habitats. For instance, there is divergence in infection patterns in populations dwelling in forested habitats compared to those in savannah (Bezjian *et al*., 2008). The biogeography, diversity and patterns of infection are also subject to abiotic variables where the levels of temperature, humidity, and moisture select for species adapted for those conditions (Appleton and Brian, 1995; Ocaido *et al*., 2003). For example it has been found that combination of cool climatic conditions and high population density tend to favor higher prevalence compared to low density baboon populations living in arid dry climate (Ghandour *et al*., 1995; Bezjian *et al*., 2008). Similarly, helminths species diversity is minimal in baboons inhabiting dry environments compared to those in wet habitats (Appleton and Brain, 1995; Muller-Graf *et al*., 1996).

2.2.2: Helminths in Vervet Monkeys

Helminth infection in vervet monkey is not adequately studied though the few studies available suggest that they harbor rich helminths fauna. Some of the helminths species identified from various populations in Africa include *Strongyloides fuelleborni, Streptopharagus sp., Trichuris sp., Primasubulura sp., Spirurina fam.gen.sp. Dicrocoeliidae sp., Physaloptera sp., Necator sp., Oesophagostomum sp*. and some unidentified strongyles (Gillespie *et al*., 2004; Legesse and Erko, 2004; Ekdahl, 2005; Kooriyama *et al*., 2012).

Vervet monkeys generally harbor more species of helminths compared to co-occuring monkeys. This was observed in Kibale National Park (Gillespie *et al*., 2004) and in Segera Ranch, Laikipia in Kenya, where vervet monkey population had higher helminths prevalence and gerater diversity compared to the sympatric population of Patas monkey (*Erythrocebus patas*) (Ekdahl, 2005). Factors that influence transmission and infection pattern of helminths in vervet monkeys are thus variable but which interact with host-parasite attributes as well as biotic and abiotic components of the habitats.

2.2.3: Helminths in Impala

Although, impala is a browser, they tend to feed on fallen leaves, seed pods of dicot trees, foliage and woody parts of the plants, which may become infectious with helminth propagules (Negovetich *et al*., 2006). As such, impala harbor a great diversity of nematodes that include *Cooperia, Oesophagostomum, Bunostomum, Cooperiodes, Gaigeria, Gonglyonema, Impalaia, Haemonchus, Longistrongylus, Muellerius, Pneumostrongylus, Strongyloides, Trichostrongylus, Trichuris, Protostrongylus,*

Muellerius capillaris and *Bigalkenema curvispiculum* (Horak 1980; 1981; Gibbons, 1973; Boomker *et al*., 1986; Ocaido *et al*., 2004). Impala also harbor diverse trematodes that include *Fasciola, Schistosoma, and Paramphistomum* (Horak, 1980; Conradie, 2008). The cestode species that have been identified in impala include *Echinococcus granulosus*, *Stilesia hepatica*, *Cysticercus* and *Moniezia* spp. (Horak, 1980; Conradie, 2008).

In Kenya, impala populations in five different sanctuaries (Lake Nakuru National Park, Lewa conservancy, Ol Jogi game reserve, Ol Pejeta conservancy, and Kongoni game reserve) varied in levels and patterns of helminth infection (Ezenwa, 2004a). The animals harbored nematodes that include strongyles, *Strongyloides sp., Trichostrongylus* and *Protostrongyloides sp.;* and two cestodes; *Thysaniezia sp.* and *Moniezia sp*. (Ezenwa, 2004a; Vanderwaal *et al*., 2014). Strongyles were the most common and persistent helminth which occured at high prevalence among impala populations in Kenya (Ezenwa, 2004b; Vanderwaal *et al*., 2014).

2.2.4: Helminths in Grant's gazelles

Very limited information is known about the helminths of Grant's gazelle except few incidences of some rare helminth species such as *Bigalkenema curvispiculum, Pneumostrongylus calcaratus*, *Hamulonema sp., Protostrongylus sp., Trichuris sp., Strongyloides sp*. and *Capillaria sp.* (Gibbons, 1973: Boomker *et al*., 1986; Ezenwa, 2003; Hoberg and Abrams, 2008). Some few studies in Kenya suggest that they can harbor very high nematode prevalence (Ezenwa, 2003; Ezenwa, 2004b).

2.2.5: Helminths in Thomson's Gazelles

Thomson's gazelle is susceptible to a great diversity of helminth species which includes nematodes, trematodes and cestodes. The diversity of nematodes Thomson's gazelle includes *Trichuris spiricollis, Haemonchus contortus, Trichostrongylus probolurus, Gazellofilaria tanganyikae, Cooperioides antidorca; Paracooperia serrata; P. daubneyi; Longistrongylus meyeri; Impalaia nudicollis; Gazellostrongylus lerouxi and Protostrongylus gazellae, Pneumostrongylus calcaratus, Bigalkenema curvispiculum Trichostrongylus* spp., *Skrjabinema* spp., and *Strongyloides* spp. (Liang-Sheng, 1956; Gibbons, 1973; Boomker *et al*., 1986; Ezenwa, 2003; 2004b; Vanderwaal *et al*., 2014). The trematode that has been identified in Thomson's gazelles is *Paramphistomum microbothriyn* while cestode is *Taenia hydatigena* (Liang-Sheng, (1956).

2.2.6: Helminths in Wildebeests

Wildebeest have a great diversity of helminths species which includes nematodes, trematodes and cestodes. The nematode diversity includes *Agriostomum, Cooperia, Gaigeria, Dictyocaulus, Oesophagostomum, Protostrongylus, Trichostrongylus, Haemonchus, Strongyloides, Trichuris, Bigalkenema curvispiculum, Pneumostrongylus calcaratus* (Gibbons, 1973; Boomker *et al*., 1986; Conradie, 2008). The trematodes include *Calicophoron, Fasciola* and *Schistosoma* while cestodes include *Taenia, Moniezia, Echinococcus, Avitellina* and *Stilesia* (Conradie, 2008).

2.2.7: Helminths in Cattle

In most parts of sub-saharan Africa, pasture ranges for pastoralist livestock usually overlap with that of wild grazers, which creates opportunity for cross-infection of

diseases, especially faecally-transmitted helminths. The common nematodes found in cattle in different parts of Africa includes *Oesophagostomum sp., Trichostrongylus sp., Bunostomum sp., Strongyloides sp., Cooperia sp., Toxocara sp., Trichuris sp.,* unidendified spirurid and *Dictyocaulus sp.* (Ocaido *et al*., 2004; Vanderwaal *et al*., 2014). Trematodes that have been identified in cattle include *Paramphistomum sp., Fasciola sp.,* and *Dicrocoelium sp*; cestodes included *Moniezia sp.* and *Stilesia sp.* (Ocaido *et al*., 2004; Pfukenyi *et al*., 2007; Nnabuife *et al*., 2013). Prevalence of helminths in free- ranging cattle varies across regions in Africa, though several population tend to have less than 60% (Swai *et al.*, 2006; Pfukenyi *et al*., 2007; Nnabuife *et al*., 2013; Vanderwaal *et al*., 2014).

2.2.7: Helminths in Goats

Helminths of veterinary importance in goats are nematodes, which includes superfamilies *Trichostrongyloidea, Strongyloidea, Metastrongyloidea, Ancylostomatoidea, Rhabditoidea, Trichuroidea, Filarioidea, Oxyuroidea, Ascaridoidea* and *Spiruroidea* (Zajac, 2006; Ocaido *et al*., 2004). *Haemonchus contortus*, *Teladorsagia circumcincta*, *Trichostrongylus* spp. *Oesophagostomum* spp., *Bunostomum sp., Strongyloides* sp., *Cooperia* spp., *Toxocara sp., Trichuris* sp., *Skrjabinema ovis* are some of the most common and harmful strongylid nematodes to goats in Africa (Bakunzi, 2003; Zajac, 2006; Mekonnen, 2007). The trematodes that infect goat are mainly the digeneans which include the liver fluke species, *Fasciola hepatica, F. gigantica* and *Dicrocoelium spp*. as well as the rumen fluke, *Paramphistomum sp.* (Urquhart *et al*., 1996; Ocaido *et al*., 2004). Goats are also significantly harmed by cestodes, especially those in the family *Taeniidae,* which specifically include cystic or larval stages of *Echinococcus granulosus*,

Taenia hydatigena and *T. multiceps* (Mekonnen, 2007). Other cestode species, whose adults cause great harm in African goats, includes *Moniezia, Avitellina, Thysanosoma* and *Stilesia* (Urquhart *et al*., 1996).

2.2.9: Helminths in Sheep

According to Mekonnen, (2007) upto 95% of the sheep in the tropics are infected with helminths of which *Haemonchus* and *Trichostrongylus* are the most problematic (Ng'ang'a *et al*., 2004; Mekonnen *et al*., 2007; Mbuh *et al*., 2008). Sheep share most of the helminths with goats, which include *Trichostrongylus Haemonchus, Oesophagostomum, Strongyloides* and *Bunostomum* (Maichomo *et al*., 2004; Kumsa *et al*., 2011).

2.3: Helminth Ecology and Epidemiological parameters

2.3.1 Parasite Prevalence

Prevalence is an epidemiological parameter referring to the proportion of the infected samples or hosts with a particular parasite or pathogen (Chapman *et al*., 2005). There are multiple factors suggested to influence prevalence. For instance, changes in climatic conditions alter landscape or vegetation structure which has drastic effects on parasite growth and transmission. Thus, it is expected that parasite prevalence could vary between habitats or vegetation types (Taylor *et al*., 2005). However, such postulation was tested in a population of Asian elephants and failed to show a significant relationship between habitat or vegetation type and prevalence (Vidya and Sukumar, 2002). Similarly, Hulbert and Boag, (2001) did not find significant difference in prevalence in sub-populations of hares inhabiting variable landscape structures.

Generally, it is expected that wet season supports growth and transmission of parasite propagules (infective eggs and larvae). This follows that parasite propagules are at risk of desiccation in dry season and therefore low transmission is expected. However, that trend is not a rule. For example, higher helminth prevalence has been recorded in Asian elephants during dry seasons compared to wet seasons (Vidya and Sukumar, 2002) which contradicts the general theory that adult worms scale down egg output in adverse climatic conditions to minimize unsuccessful transmission. This suggests that other than just seasonal conditions, there are other factors that regulate prevalence such as host immunity, gregariousness and density (Moller *et al*., 1993; Nunn *et al*., 2003).

2.3.2 Abundance

Abundance refers to the mean number of parasites in an infected host (Altizer *et al*., 2003). The ideal method of determining intensity of infection is to quantify adult worms. However, access to adult worms is through necropsy, which is an invasive and most often destructive procedure, hence deemed unethical for wild hosts (Taylor *et al*., 2005). An alternative approach is to quantify eggs, absolute count (Gillespie, 2006) or by eggs per gram (epg) to infer worm intensity or burden. However, these indices ought to be interpreted with caution because most studies have failed to show correlation between the number of adult worms and egg output (Shaw and Dobson, 1995; Taylor *et al*., 2005) suggesting that such indirect methods are inadequate methods for assessing parasite abundance within host gastrointestinal tract.

2.3.3 Parasite Species Richness

Parasite species richness (PSR) or diversity refers to the number of all parasite species identified from a given host species (Morand, 2000). Parasite richness is influenced by host body size, host density and geographical range (Arneberg, 2002; Ezenwa *et al*., 2006; Lindenfors *et al*., 2007). For instance, the size of a host home range is thought to drive PSR because parasite propagules depend on host availability or mobility for dispersal and transmission. It is therefore, argued that animals with larger home ranges are likely to encounter more diverse parasite propagules or potentially parasite infested/infected hosts leading to high PSR (Nunn *et al*., 2003; Lindenfors *et al*., 2007). Contrary to this prediction, Nunn *et al*., (2003) and Bordes *et al*., (2009) found negative correlation between home range and PSR especially for helminths. Ezenwa *et al*., (2006) did not find correlation between home range and PSR among ungulates. An alternative theory by Bordes *et al*., (2009), suggests that contrary to assumptions that expansive home ranges enhances parasite encounter, it may rather be a host strategy to avoid or limit infection as explained by the concept of migratory escape practiced by reindeers, fish, monarch butterflies and baboons (Hausfater and Meade, 1982; Folstad *et al*., 1991; Bartel *et al*., 2011; Poulin *et al*., 2012).

2.4 Methods of helminth identification

The ideal method to determine species diversity and infection burden of helminth in a host is to recover mature stages. The procedure for recovering mature helminths from a host is invasive, host destructive and unethical, hence not acceptable in many parts of the world, including Kenya. A basic approach that is recommended because it is non-invasive is the use of coproscopic parasitological methods: floatation and sedimentation

techniques. Coproscopy is based on microscopic examination for distinctive phenotypic features of the parasite propagules present in faecal samples. These methods are inherently time consuming and requires combination of taxonomic skills and experience. A major inadequacy of coproscopic methods is that it cannot distinguish to the lowest taxa those parasite propagules that are phenotypically identical (Harmon *et al*., 2006). Specifically, these techniques are insensitive and unspecific for differentiating eggs and larvae of strongylate nematodes in the superfamily: Strongyloidea, Ancylostomatoidea and Trichostrongyloidea (Hoberg *et al*., 2001). Yet, a superfamily like Trichostrongyloidea, which has the greatest taxonomic, genealogical and numerical diversity and also constitutes potential pathogenic nematodes (Hoberg and Lichtenfels, 1994) of medical and veterinary importance, needs to be well understood and continually reviewed. Although L3 larvae of some nematoes can be microscopically identified, it is a challenge when working with wildlife samples because there is lack of reliable reference for identifying helminths in wildlife.

The inadequacies of coproscopic methods have been overcome by molecular analytical tools, especially genetic techniques which since their advent have greatly improved understanding of helminth epidemiology, biogeography and phylogeny. For instance, genetic techniques are increasingly being used to address complex questions in parasitism such as gene flow, genetic variations and differentiation of intra-specific strains as well as discovery of cryptic species (Zarlenga *et al*., 1999; Eysker and Ploeger, 2000; Archie and Ezenwa, 2011; Ghai *et al*., 2014). More interestingly, genetic techniques offer opportunity to understand how generalist nematodes (species capable of infecting

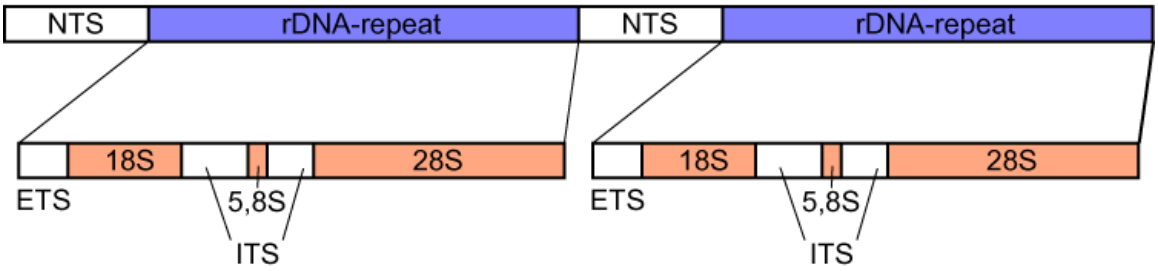
multiple host taxa) such as *Oesophagostomum bifurcum* or *Trichostrongylus axei* distribute themselves among sympatric hosts.

2.5 Genetic techniques for identification of helminths

Advances in DNA technology have led to the development of sensitive and specific methods for the identification of helminths. The techniques are sensitive to samples which can be acquired non-invasively such as eggs and larvae (Zarlenga *et al*., 1998; 1999). This means that these techniques are quite ideal for studying helminthosis in all animals but specifically more appropriate for wild animals since invasive approach would mean culling of the animals to extract adult worms for identification, which are both illegal and unethical procedures in Kenya.

Genetic techniques require initial definition of one or more suitable DNA target regions (genetic marker or locus). Since different genes evolve at different rates, the selected region should be highly variable in sequence to differentiate the helminths to the required taxonomic unit (Roeber *et al*., 2013). It is required that there should be no (except minor) sequence variations in the target gene within a species for it to effectively delineate among different species (Roeber *et al*., 2013). However, when targeting to pick variants ('strains' or genotypes) in a population, the sequence of the target gene should have high level of variation within the species (Roeber *et al*., 2013). As such, several regions of the nuclear and mitochondrial genes have been identified as reliable markers for species or sub-species identification of helminths (Blouin, 2002; Chilton, 2004; Gasser, 2006).

Since there are very low sequence variations in the nuclear ribosomal genes and spacers among individuals within a population and between populations, they are frequently used as species-specific markers for helminths and other invertebrates (Roeber *et al*., 2013). The Internal transcribed spacer (ITS) region of ribosomal DNA (rDNA), in particular ITS-1 and ITS-2 (Figure 1), are non-coding regions located in rDNA between 18s and 28s of the rRNA gene respectively (Jansen *et al*., 2006; Won and Renner, 2005).



Author: Norbert Holstein, 2006.

Figure 2.10 : Schematic drawing of the gene segment of the nuclear ribosomal (rDNA) repeat units consists of three coding tracts; 18S, 5.8S, and 28S tracts and the non-coding internal transcribed spacers. Key: NTS - non-transcribed spacer; ETS - external transcribed spacer; ITS -internal transcribed spacers 1 and 2.

These regions are commonly used to distinguish closely related species of helminths including between strongylid nematodes (Chilton *et al*., 1995; Hung *et al*., 1996). The ITS, as a genetic marker, is quite sensitive even for small quantities of samples such as a single egg and has been used to differentiate between *Cooperia, Haemonchus, Trichostrongylus, Nematodirus* and *Ostertagia* genera (Schnieder *et al*., 1999; Harmon *et al*., 2006; Gasser *et al*., 2008). In addition the popular use of ITS genes is because they are short (≤ 800 base pairs), repetitive and undergo homogenization (Elder and Turner, 1995; Gasser, 2006), factors that underpins specificity, efficiency and sensitivity of any

amplification process (Roeber *et al*., 2013). The ITS genes are also useful for picking up cryptic species (morphologically similar but different genetically) in a population (Gasser and Chilton, 2001; Gasser *et al*., 2006).

Mitochondrial DNA (MtDNA) is another genetic marker frequently used for species identification and phylogenetic analyses of helminths. McLean *et al*., (2012) used the gene for identification of nematodes in African elephants and it was remarkably successful in differentiating closely related species. Archie and Ezenwa, (2011) also used mtDNA to identify and explain the genetic structure of *Trichostrongylus axei* in a multi-host system and their results clearly indicated fine scale population genetics of the species. Further, McDonnell *et al*., (2000) also used mtDNa to unravel the phylogenetic relationships among the nematodes in the complex family of Cyathostominae. The DNA in the mitochondria contains 13 protein coding genes, two ribosomal genes and a D-loop (Figure 2.11). This gene is in popular use for species identification because it undergoes rapid evolution, a trait that enables it to discriminate not only between closely related species but also phylogeographic groups within a single species (Cox and Herbert, 2001; Wares and Cunningham, 2001). In addition, it is highly efficient in picking up potential cryptic species from small number of individuals. In summary, both ITS and MtDNA are useful genetic markers for studying the epidemiology of helminthosis and their combined application in a study adds their robust traits to the results. Specifically, ITS genes are excellent tool for diagnostic identification whereas, mtDNA is excellent for molecular prospecting, especially detecting cryptic species (Blouin, 2001).

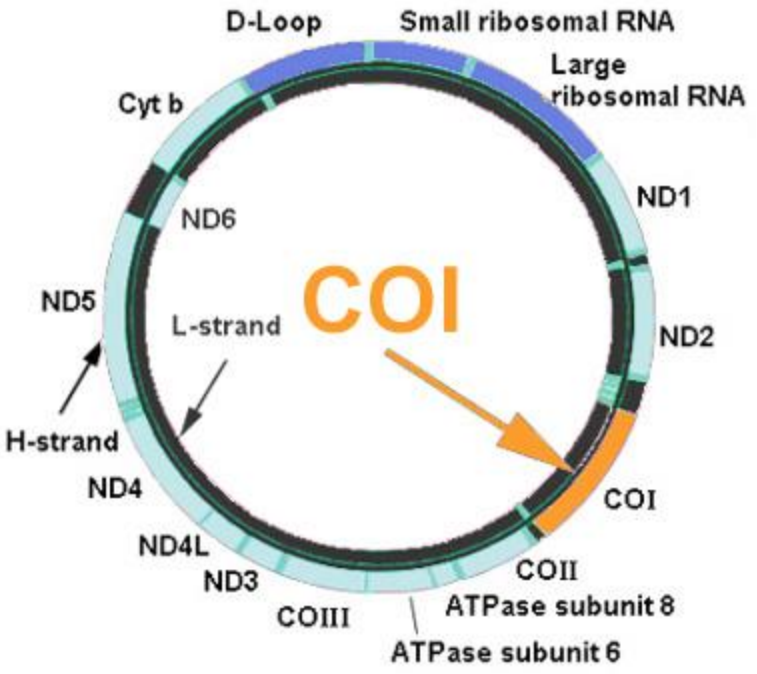


Figure 2.11: Graphical scheme of Mitochondrial DNA showing the position of 13 protein-coding genes, two ribosomal genes, a D-loop and other several genes. The large arrow point at the position of CO1 while the two black arrows show the H-strand and L-strand.

Source:http://www.dnabarcoding.ca/primer/images/mito_genome_large.jpg.: Accessed 12/12/2014

2.6 Habitat range overlap

The space in an animals' habitat is an important component of their ecology and how they use it affects their relationships with conspecifics as well as heterospecifics, especially in terms of social structure, mate searching and specifically human-wildlife conflicts (Pearce *et al*., 2013). Space use also has implications in diseases or pathogen spread because it regulates contact rates between infected hosts and between host and parasites. This space that is regularly used by a particular species for its natural activities

in the course of a year is referred to as habitat- or home-range (Burt, 1943). Factors that influence the use and size of home range are multiple and their interactions are likely to be complex but most definitely vary with animal species and covary with habitat quality (McLoughlin *et al*., 2000; McGill, 2008): animals in rich habitats have small exclusive home ranges whereas those in poor areas have large overlapping habitats (South, 1999; McLoughlin *et al*., 2000). Size of home ranges has been seen to be positively influenced by seasonality whereas home range overlaps also vary with seasonality but in a non-linear manner (McLoughlin *et al*., 2000).

Home range is one of the measures of how animals use space within their habitats (McNab, 1963) and can be determined by several methods such as Minimum Convex Polygon (MCP), Fixed or Adaptive Kernel, Bivariate Normal or Fourier but each method varies in strengths and weaknesses (White and Garrott, 1990; Harris *et al*., 1990). Home range by MCP is derived by geo-referencing all location points of individuals for a period and then mapping out the outline using appropriate software. When all the outer location points are included, it represents the entire space used by the animal, hence 100% MCP. However, animals tend to use particular smaller area within their home range and proportion of this can be determined, at either 50% or 75% MCP. The advantage of MCP over other statistical methods (Fixed or Adaptive Kernel, Bivariate Normal or Fourier) of deriving home ranges is that it can accurately represent home range at sightings >100 (Ruby and Dunham, 1987; Worton, 1989; 1995; Seaman and Powell, 1996).

2.7 Indices for animal presence and population estimation

Several methods have been derived to estimate population abundance of wild animals. The choice of method must take cognizance of repertoire of animal behavior and habitat

preference. For example, some species are nocturnal hence methods must suit night conditions. Nevertheless, fundamentally there are two methods: direct and indirect sampling techniques.

2.7.1 Direct sampling method

The direct sampling methods are commonly applied in population census exercises and include road and field strip counts (Dinerstein, 1980; Underwood, 1982) drive counts (Bothma *et al*., 1990), waterhole counts (Bothma *et al*., 1990), aerial counts (Reilly, 2002) and line transects (Lannoy *et al*., 2003). Each method works best for particular habitats and type of animal species.

Drive counts involve being in a vehicle driven in a straight line from one boundary of the conservation area to the other end while counting observed animals or target species (Bothma *et al*., 1990; Bothma, 2001). This method is recommended for large animal species residing in open savanna grassland (Bothma, 2001).

Waterhole counts involve observation and counts of all animals or target species simultaneously in all the waterholes in the conservation area during a continuous 24 hour period (Bothma *et al*., 1990). Aerial surveys involve observing and counting animals while on aircraft that flies predetermined transects over the conservation area (Bothma, 2001).

Road and field strip counts involves vehicle driven on a designated road while counting encountered animals or target species. However, in a road strip, the mean visibility from the road is predetermined and used as the width of the strip hence all animas visible on each side of the road are enumerated (Dinerstein, 1980; Bothma, 2001). Field strip

method is based on a predetermined area and the observer counts all the animals that cross the area during a particular period (Bowland and Perrin, 1994). Most of these methods are based on the assumptions that no animal is counted more than once or no animal moves out of range before detection (Schmidt, 1983; Bowland and Perrin, 1994). There are several variations to strip methods. For example, the line-transect technique involves measurement of the perpendicular distance from the line to the sighted animal (Bowland and Perrin, 1994).

2.7.2 Indirect sampling methods

The behavior of some animal species and to some extent the habitat type does not allow use of direct methods or render their use inaccurate, hence several indirect methods have been developed as indices of presence or counts. These methods, such as dung/pellet counts (Dinerstein, 1980; Schmidt, 1983; Bowland and Perrin 1994), territorial marking (scrapes or rubs) counts (Llaneza *et al*., 2014), track counts (Mandujano and Gallina, 1995; Mayle *et al*., 2000), have been widely used to infer animal species presence, yet they vary in strengths and weaknesses. The basis of these techniques is that these signs signify presence of particular animals and counts of these signs may represent actual demography.

The use of dung/pellets counts as an index of animal presence is advantageous in that it can be used to estimate mean abundance over a period unlike in direct counts in which estimates are based on a single day count (Marques *et al*., 2001). According to Putman, (1984) pellet/dung counts generate a richer data set on population dynamics, such as population size and spatial distribution especially for the elusive species.

Dung or pellet counts have been used to estimate population size of multiple species of small- and mega-sized mammals such as elephants and gorilla (Barnes, 2001; Takenoshita and Yamagiwa, 2008). However, Aulak and Babillska-werka (1990) recommend determination of defecation rate of the animal and decay rate of the dung/pellet when calculating density. Since defecation rate is influenced by multiple factors such as dietary composition, season, individual animal intrinsic factors, Takenoshita and Yamagiwa (2008) suggest that abundance based on dung pile counts should not be generalized across multiple species.

Counting dung pile may be based on standing crop method, in which all dung/pellet piles within the transect strip plots are counted, or clearance plot method, which is based on counting new dung deposits on the plot that was initially cleared of all dung (Marques *et al*., 2001). Transect plots may be shaped variably such as in form of circular, quadrat or rectangular (Dinerstein, 1980, Bowland and Perrin, 1994; Takenoshita and Yamagiwa, 2008) whereas size of transect is recommended to be small. For instance, Marques *et al*., (2001) recommends plots of 1-2 m wide plots in case of strip rectangular plots, since the strip can be covered in a single day.

2.8 Effects of habitat overlap on helminth transmission

The precursor of parasite transmission and disease is contact between susceptible hosts, hosts and vectors or hosts and infectious parasite life-stages. This implies that contact is an essential fundamental determinant of transmission hence understanding factors that influences it is crucial in disease ecology as well as in disease management (Williams *et al*., 2014). In free ranging systems, it is a challenge to observe or directly measure contacts between individuals or groups, hence contact rates tend to be inferred from

metrices such as the degree to which two habitats of individuals, species or groups, overlap (Minta, 1992; Roemer *et al*., 2001; Schauber *et al*., 2007; Jiménez, 2007). Determining areas of habitat overlap requires sufficient GPS data points of animal locations and should include both wet and dry seasons because home range size and home range overlaps vary seasonally (McLoughlin *et al*., 2000). This implies that contact rates will definitely differ seasonally (Williams *et al*., 2014). It is predicted that high overlap in space use, probably inferred by the size of the overlapped area, signifies increased opportunity for host-parasite contacts, though this assumes that animals contact each other randomly and occupy the space randomly (Williams *et al*., 2014). This notion has led to the prediction that areas of great biodiversity overlap are hotspots for cross-transmission and consequently potential areas for emergence of new pathogens or diseases (Page, 2003; Daszak *et al*., 2008; Jones *et al*., 2008). The prediction of disease hotspots is based on the fact that habitat overlap and host phylogeny creates unprecedented opportunity that (1) drives cross-transmission especially among closely related hosts and (2) allow pathogens to adapt for host-shifts, eventually leading to host range expansions (Antonovics *et al*., 2002; Davies and Pedersen, 2008).

Parasite sharing is thus predicted to be common in areas where multiple hosts overlap. Viruses and bacteria top the list of pathogens that co-occur in sympatric host community especially those that jump to new hosts. The latter case is because viruses and bacteria undergo rapid mutations that get established in the progeny due to short generation turnaround.

CHAPTER THREE: DEGREE OF HABITAT OVERLAPS AMONG BABOON GROUPS AND BETWEEN BABOON AND ALTERNATIVE HOSTS IN AMBOSELI ECOSYSTEM, KENYA

3.1 Introduction

One of the goals of community ecology is to determine factors that promote co-existence of multi-species host assemblages that often differ in many variables including niche, trophism and space use (Hopf, 1993; Darmon *et al*., 2012). The co-occurrence of multiple hosts in a particular area of the habitat implies that they have common factors that determine their preference or selection for that site. Habitat selection by free-ranging grazers is subject to resource availability, which includes quality of pasture and water. However, in nature, resource availability and spatial distribution is often heterogeneous and both quality and quantity fluctuates with seasonality (Darmon *et al*., 2012). This means that as resources vary spatially and seasonally, the level of habitat occupancy or interaction by sympatric animals varies across landscapes and between seasons (Sundell *et al*., 2012).

According to Wehtje and Gompper (2011), when habitat resources needed by animals are spatially clumped in a habitat, it leads to local animal aggregations in such sites or habitats, which may lead to either tolerance or varying levels of competition. When animals are highly aggregated, their habitat or home ranges tend to become highly overlapped. For example, the natural swamps that occur in Amboseli National Park is an oasis that provides lush vegetation and water year round, such a resource is a key driver to shaping the patterns of animal distributions as well as levels of habitat overlap (Chiyo *et al*., 2014). Moreover, seasonal changes in resource abundance results in temporal changes in host preference for a given habitat area thereby influencing seasonal shifts in

habitat selection by herbivores (Zweifel-Schielly *et al*., 2009; Bennit *et al*., 2014) and baboons (Barton *et al*., 1992). The relationships between overlapping host species may be neutral, because either they need dissimilar resources, resources used in common are not limited, or species-specific disparity in responses to environmental uncertainties, hinder competition (Duchesne *et al*., 2000, Manor and Saltz, 2003, Loehr *et al*., 2005; Darmon *et al*., 2012).

There are few studies that have investigated the degree of habitat overlap between or among conspecifics. For instance, elephants, being highly social animals, the degree of range overlap between social groups have been found to vary across regions (Chiyo *et al*., 2014). Specifically, elephant groups in Samburu, Kenya were larger and had higher percent overlap compared to populations in Amboseli ecosystem (Chiyo *et al*., 2014). The spring water swamp in Amboseli National Park was found to be a central resource driving overlaps among elephant social groups (Chiyo *et al*., 2014). In regard to baboons, home ranges of their social groups are often described or perceived (Muller-Graf *et al*., 1996; Ocaido *et al*., 2003; Legesse and Erko, 2004; Ebbert *et al*., 2013) as overlapped but the degree of such overlaps are unknown. Moreover, there are no records of the level of habitat overlap between baboons and other host taxa. This study aimed at determining the degree of overlap across baboon social groups and between baboons and their heterospecifics.

3.2 Material and Methods

3.2.1 Study area

The study was carried out in the Amboseli ecosystem which includes the Amboseli National Park (~392 km^2) and the communally-owned group ranches (~5000 km^2) in both the Kenyan and Tanzanian side of the border (Figure 3.1). The ecosystem is characterized by semi-arid savanna, with open grasslands mixed with patches of scrubs and *Acacia xanthophloea* woodlands.

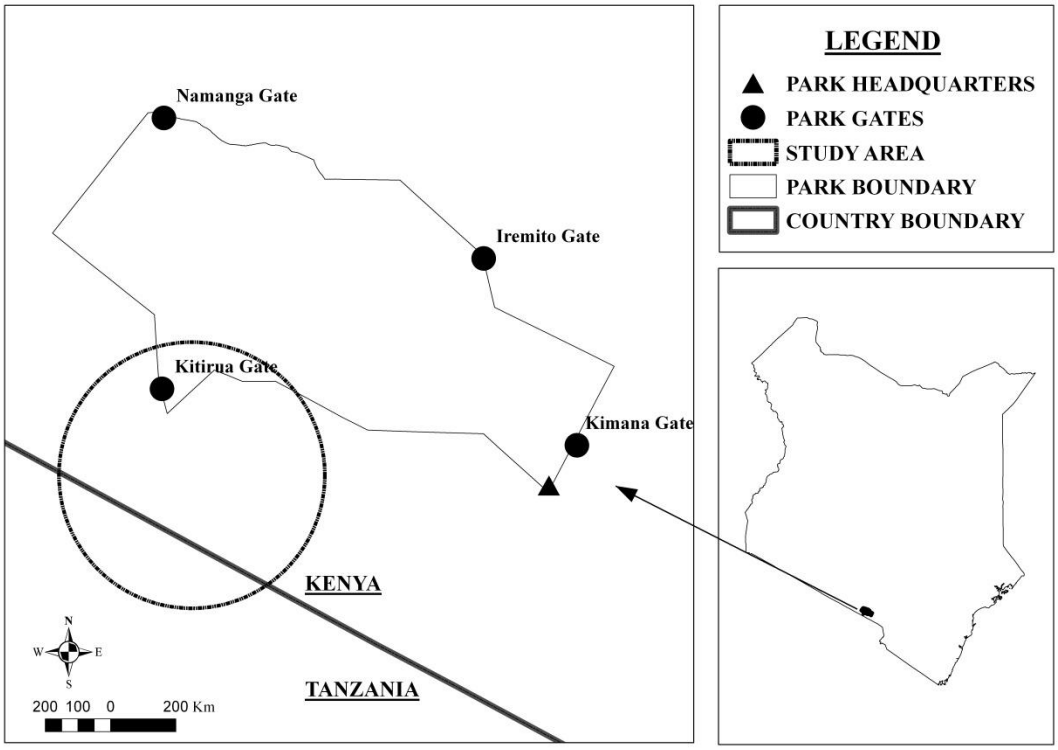


Figure 3.1: Map showing area of study, which included part of the Amboseli National Park and the surrounding community areas. Prepared by the author, 2014.

Rainfall pattern is erratic (Figure 3.2) with an average annual mean of 348mm and a range as low as 150mm to 550mm (Altmann *et al*., 2002). The long rains occur from March to May while the short rains occur during the period of November - December. Short dry season occurs in January - February and the long dry season is from June to the end of September (Figure 3.2). This region has undergone significant changes in seasons and habitat structure since early 1960s (Western and vanPraet, 1973; Altmann *et al*., 2002; Alberts *et al*., 2005). Apart from the rainfall, the region is watered by several natural springs that create pools and marsh. In addition, the Maasai community has dug out water pans to supplement water provision for their livestock.

The Maasai community who are pastoralists, dominate the area and use the zone for grazing their indigenous cattle (*Bos taurus*), sheep (*Ovis aries)*, goats (*Capra hircus*) and donkey (*Equus africanus asinus)*. This zone is also the natural habitat of diverse wildlife that includes elephants (*Loxodonta africana*), lions (*Panthera leo*), giraffes (*Giraffe camelopardalis*), plains zebra (*Equus burchelii*), impala *(Aepyceros melampus)*, Grant gazelle (*Gazella granti*), Thomson's gazelle (*Gazella thomsoni*), wildebeest (*Connochaetus gnu*), vervet monkey (*Chlorocebus aethiops*) and baboons (*Papio spp*).

The ecology and behavior of baboons in this region has been studied for over three decades (Alberts *et al*., 2005). Their social groups are well defined and individuals of each social group are known. Seasonal changes directly alter the environmental conditions as well as foraging pattern of baboons. According to Alberts *et al*., (2005), seasonality leads to baboon diet shift as wet season is characterized by lush environment (Figure 3.3) whereas dry season leads to pasture die-back (Figure 3.4).

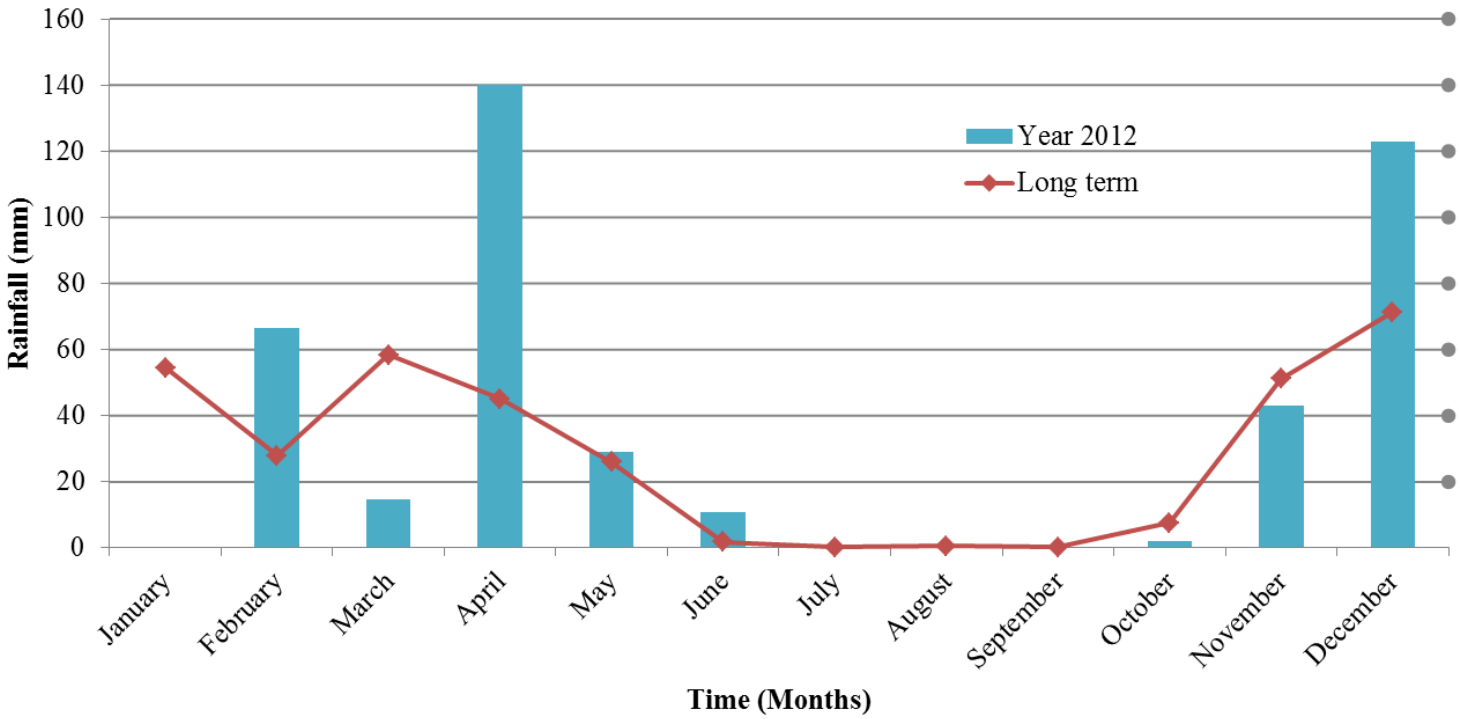


Figure 3.2: The mean monthly long term rainfall between 1990 and 2012 in Amboseli ecosystem, Kenya and the monthly rainfall in 2012 in Amboseli ecosystem. Data courtesy of the Amboseli Baboon Project based on the southern border of Amboseli National Park, Kenya.

Figure 3.3: Baboons foraging on flowers in the Amboseli ecosystem, Kenya during lush times in the rainy season

Figure 3.4: Baboons and zebra at one of the seasonal water holes during dry season in Amboseli ecosystem, Kenya

3.2.2 Study population

The species of animals included in this study are categorized as follows:

3.2.2.1 Non human primates

This group consisted of vervet monkeys (Figure 3.5A) and baboons (Figure 3.5B). In free ranging system, baboon population is socially structured hence the groups and group sizes (*n*) of baboon study population in Amboseli are well known and have been given names since they have been studied continuously for more than three decades. The total number of baboons were : Weaver (*n* =117), Hokey (*n* = 72), Viola (*n* = 64), Narasha (*n* = 43), Mica (*n* = 34) and Snap (*n* = 28). However, the population structure of vervet monkeys in Amboseli is not known, though they co-occur in sympatry with the baboons.

3.2.2.2 Wild ungulates

This group consists of Impala (Figure 3.5C), Thomson's gazelles (Figure 3.5D), Grant's gazelles (Figure 3.5E) and wildebeests (3.5F). These are medium-body sized African ungulates of the family Bovidae whose social structure is unstable but rather highly gregarious and occupy relatively small home ranges (Du Toit, 1990) across the savanna ecosystem.

3.2.2.3 Domestic ungulates

This group consisted of indeginous goats (Figure 3.5G), sheep (Figure 3.5H) and cattle (Figure 3.5I), reared by the Maasai community under nomadic pastoralism system. This means that the animals were grazed extensively across rangelands in search of pastures and water.

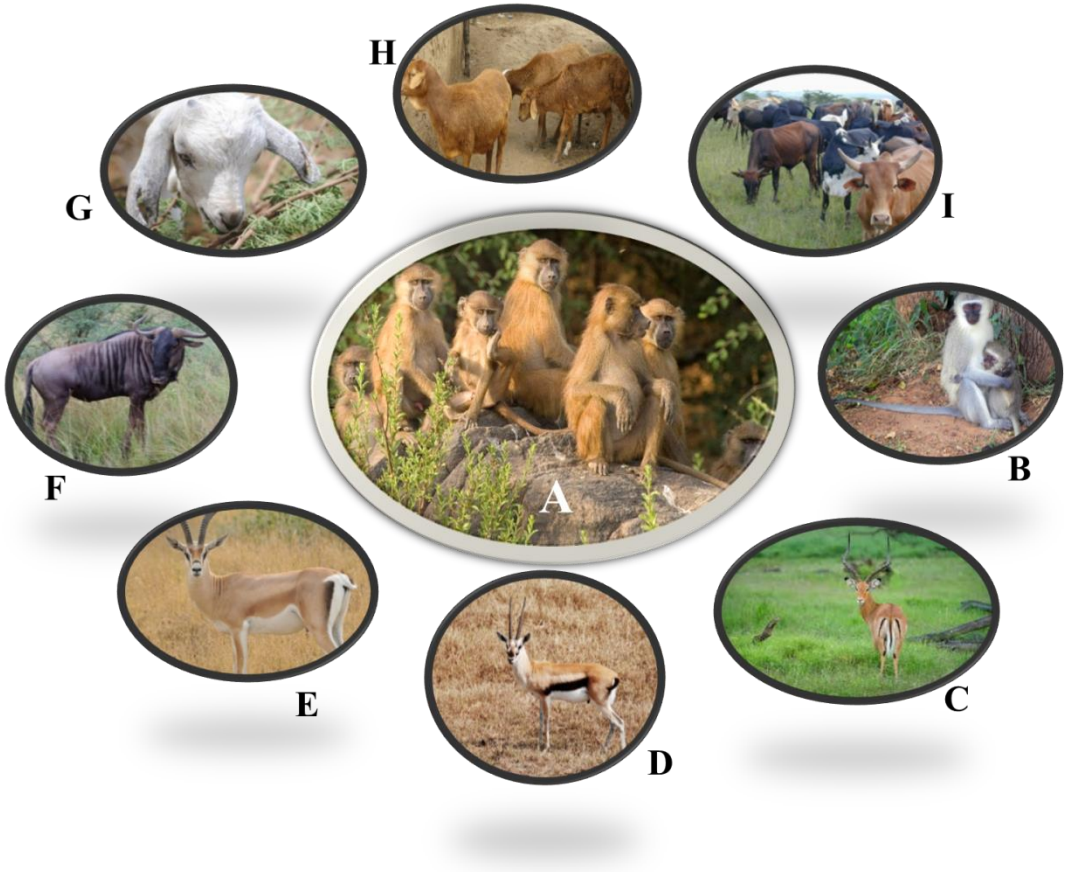


Figure 3.5: Sympatric host species that co-occur in the habitat range of baboons in Amboseli ecosystem, Kenya. The animals includes: (A) Baboon, (B) Vervet Monkey, (C) Impala, (D) Thomson's gazelle, (E) Grant's gazelle, (F) Wildebeest, (G) Goat, (H) Sheep and (I) Cattle.

3.2.3 Determination of the degree of habitat overlap among baboon social groups

Baboon social structure is usually organized in hierarchy with dominant ranked individuals (alpha males or higher ranking females) leading the troop. As such, the dominant individuals in each social groups of the Amboseli population were known and had been fitted with global system for mobile communication (GSM) collars to generate group activity. Therefore, in the present study, the collared individuals were tracked by GSM radio receiver to locate the group. Once located, these individuals were tracked daily from 6 am to 12 pm and in alternative days they were tracked from 12 pm to 7 pm, which is about the time they settle in sleeping groves. Each social group was tracked for 7 consecutive days. Data were collected in the months of January and February 2012 and October 2012, which corresponded with the wet and dry seasons. During tracking, a series of locations were geo-referenced and the coordinates recorded as waypoints in geographical positioning system (GPS) equipment (Garmin® GPS76), which was transferred to Microsoft Excel software. This procedure was followed to collect at least 100 spatial location points tracked in both wet and dry seasons. The coordinates were transferred from Excel to a home range mapping software (BIOTAS 2.0 alpha, Ecological Software solutions). This procedure was applied for all the social groups. Home range for each social group was derived by Minimum Convex Polygon (MCP) method (Worton 1989; 1995; Seaman and Powell, 1996). The MCP method was used because specifically, it could (1) determine home range (2) calculate size of the home range (3) calculate the shared area (km^2) or proportion (%) of the total area and (4) determine the number of the overlapping groups. The home ranges were determined at

100% and 50% core habitat levels. The home range area (km^2), size of the overlapped area (km^2) and number of overlapped home ranges were generated by BIOTAS software.

3.2.4 Determination of the degree of habitat overlap between baboons and other alternative hosts

Direct and indirect methods were used to estimate populations of alternative host species in each of the baboon home ranges. Data collection was carried out in January and February, 2012 which was the wet season and again was repeated in October, 2012 during dry season. The first approach was based on indirect sampling method in which dung/pellet counts were carried out as index of presence of alternative animals in baboon home ranges (Dinerstein, 1980; Schmidt, 1983; Bowland and Perrin, 1994; Marques *et al*., 2001). The counts were carried out on standing crop of pellet/dung piles within strip transects of 2 m wide and 200 m long (Marques *et al*., 2001). Location of transects was spatially distributed to ensure the home range area is sampled to the maximum. The transect start and end points were marked by GPS coordinates to fix the strip plot for repeatability in each season. The number of transects ranged between six and eight in each baboon home range. A pile of pellet was defined as dung heap of at least five or more pellets of similar shape that seems to have been deposited at the same time. A dung field guide by Stuart and Stuart, (2000) was used to assist in differentiating pellets to host species. Each transect was walked while identifying the host species associated with the dung/pellet pile while recording the GPS coordinates and number of pellets only for those within the strip plot. The 2 m width of strip plot was maintained by initially placing marks at intervals of 50 m along the plot length. The counts and GPS coordinates were

entered in Excel software and then transferred to BIOTAS software to determine the degree of overlaps.

The second approach was direct method which was based on drive counts in which encountered individuals or herds were counted and their locations geo-referenced (Bothma *et al*., 1990; Bothma, 2001). The drive counts were carried out between 6am and 3pm and in alternative days between noon and 7pm. The drive counts were carried out for 14 consecutive days in the dry and in the wet season. The GPS coordinates were entered in Excel software and then transferred in BIOTAS software to determine frequency of observation of alternative species in each baboon home range.

3.3 Results

3.3.1 Degree of habitat overlap among baboon groups

Each habitat range of baboon group measured at 100% MCP overlapped with at least four other habitat ranges of baboon groups (Figure 3.6 and 3.7). The number of habitats that overlapped in each baboon group's range was identical in both dry (Table 3.1) and wet seasons (Table 3.2). Mica was the only group that had an exclusive area (22.49%) within its habitat range but only during the dry season whereas all the other groups shared their ranges completely (Tables 3.1 and 3.2). The sizes of the overlapped area were generally larger in the wet season compared to the dry season except for Mica which was larger in the dry season (Table 3.1 and 3.2).

At 50% MCP, each habitat range of baboon group overlapped with at least one other range of habitat of another group (Figure 3.6 and 3.7). The habitats of Mica and Narasha were completely overlapped and separated from the rest (Figure 3.6 and 3.7), whereas other groups had exclusive areas of variable sizes within their habitat ranges. Hokey had the largest exclusive area (98.2%) in the dry season (Table 3.3) but the size reduced (75.77%) in the wet season (Table 3.4). In contrast, Weaver (98.5%) and Viola (91.59%) were the two groups that had the largest exclusive areas of their home ranges in the wet season (Table 3.4). Except for Hokey, the overlapped areas were generally smaller in wet season compared to dry season (Table 3.3 and 3.4), which was in contrast to observations at 100% MCP habitat level.

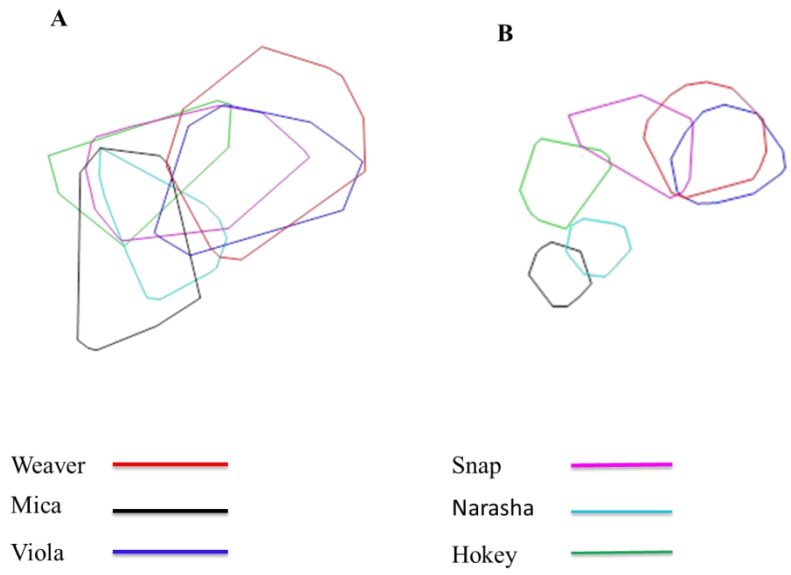


Figure 3.6: Habitat range overlaps measured at 100% MCP (A) and 50% MCP (B) during dry season among the six social groups of baboons.

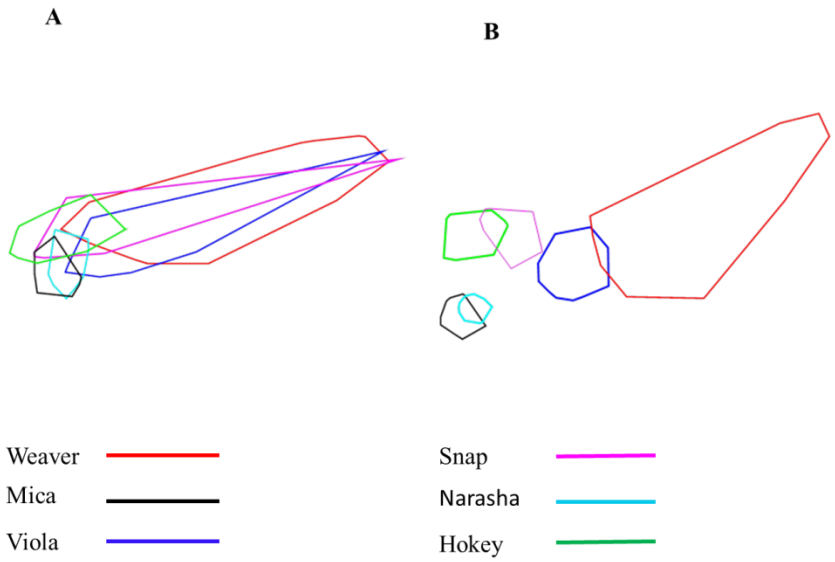


Figure 3.7: Habitat range overlaps at 100% MCP (A) and 50% MCP (B) during wet season among the six social groups of baboons.

Table 3.1: Size of habitat and overlap area ($km^{2)}$ between baboon social groups derived at 100% MCP in the dry season.

Baboon groups	Size in dry season (km^2)		Exclusive habitat (%)	Shared habitat (%)	Number of overlaps
	Habitat	Overlap area			
Hokey	7.77	15.89	0	100	5
Mica	10.45	8.1	22.49	77.51	4
Narasha	5.9	7.87	0	100	4
Viola	11.43	20.5	0	100	5
Weaver	15.59	18.7	0	100	5
Snap	11.67	26.53	0	100	5
Mean	**10.47**	**16.27**		**96.2**	**4.67**

Table 3.2: Size of habitat and overlap area ($km^{2)}$ between baboon social groups derived at 100% MCP in the wet season.

Baboon groups	Size in wet season (km^2)		Exclusive habitat (%)	Shared habitat (%)	Number of overlaps
	Habitat	Overlap area			
Hokey	12.06	55.38	0	100	5
Mica	5.12	5.43	0	100	4
Narasha	5.09	8.29	0	100	4
Viola	33.4	52.29	0	100	5
Weaver	57.61	96.05	0	100	5
Snap	32.1	57.65	0	100	5
Mean	**24.23**	**45.84**		**100**	**4.67**

Table 3.3: Size of habitat and overlap area (km^2) between baboon social groups derived at 50% MCP in the dry season.

Baboon groups	Size in dry Season (km^2)		Exclusive habitat (%)	Shared habitat (%)	Number of overlaps
	Habitat	Overlap area			
Hokey	2.16	0.04	98.2	1.8	1
Mica	1.1	3.2	0	100	1
Narasha	1.1	3.2	0	100	1
Viola	3.31	2.93	11.5	88.5	2
Weaver	4.2	3.77	10.2	89.8	2
Snap	3.2	1.58	50.6	49.4	3
Mean	**2.51**	**2.45**		**71.5**	**1.67**

Table 3.4: Size of habitat and overlap area (km^2) between baboon social groups derived at 50% MCP in the wet season.

Baboon groups	Size in wet season (km^2)		Exclusive habitat (%)	Shared habitat (%)	Number of overlaps
	Habitat	Overlap area			
Hokey	2.6	0.63	75.77	24.23	1
Mica	1.29	0.35	72.87	27.13	1
Narasha	0.7	0.35	50	50	1
Viola	4.16	0.35	91.59	8.41	1
Weaver	23.3	0.35	98.5	1.5	1
Snap	2.56	0.63	75.39	24.61	1
Mean	**5.77**	**0.44**		**22.6**	**1**

3.3.2 Degree of overlap between baboons and alternative host species

3.3.2.1 Dung piles as an index of overlap

Dung pile counts of alternative hosts were used to infer the degree of overlap between them and baboons. Tables 3.5 and 3.6 show the variation in degree of overlap between alternative hosts and baboon social groups' within their core area of habitat (50% MCP) and maximum area of habitat (100% MCP) as well as between seasons.

The degree of overlap (mean dung pile count) in 50% MCP of the baboon range was smaller (25.6) compared to the overlap in 100% MCP, which was 94.2 (Table 3.5 and 3.6). At 50% MCP habitat range (Table 3.5), Hokey was the baboon group with the highest overlap (mean dung pile count at 46.8) while Narasha had the least overlap (mean dung pile count at 8.3). At 100% MCP habitat range (Table 3.6), Snap was the group with the highest overlap (mean dung pile count at 137). The baboon group that consistently had the least overlap in its habitat at both 50% MCP and 100% MCP ranges was Narasha (Table 3.5 and Table 3.6).

Host taxa that greatly overlapped with baboons in both 50% MCP (mean dung count pile at 72.1) and 100% MCP (mean dung count pile at 267.7) of habitat level (Table 3.5 and 3.6) were cattle. The host species that showed least overlap in baboon ranges in both 50% MCP and 100% MCP with mean dung pile counts of 1.4 and 4.6 respectively, was impala (Table 3.5 and 3.6). Since dung piles of vervet monkeys tended to be clustered around their sleeping groves (Obanda. V., *personal observation*) rather than being randomly distributed in the habitat, the transect counts of their dung piles would have been biased and thus were not included in the analysis. However, for the other animals, their faecal were not clustered, rather randomly occurring in the environment.

Table 3.5: Dung pile counts of alternative host species in baboon group's habitat ranges at 50% MCP.

Dung counts at 50% MCP in wet and dry seasons													
	Weaver		**Viola**		**Snap**		**Narasha**		**Mica**		**Hokey**		
Hosts	**Dry**	**Wet**	**Dry**	**Wet**	**Dry**	**Wet**	**Dry**	**Wet**	**Dry**	**Wet**	**Dry**	**Wet**	**Mean**
Cattle	100	57	119	33	37	70	17	15.8	24	75	131	138	**72.1**
Goat	11	0	16	1	0	29	0	18	3	33	77	54	**20.2**
G.gazelle	33	86	26	10	26	55	1	19	20	18	14	78	**32.2**
Impala	1	0	11	0	1	2	0	0	0	0	0	2	**1.4**
Sheep	3	0	12	2	0	17	1	10	0	23	53	13	**11.2**
T.gazelle	8	16	5	0	9	39	0	0	5	0	8	30	**10**
Wildebeest	93	129	55	1	17	44	0	0	0	0	4	53	**33**
Season mean	36.6	41.4	34.8	6.7	12.8	36.6	2.7	13.9	7.4	21.2	41	52.6	
Group mean	**39**		**20.7**		**24.7**		**8.3**		**14.3**		**46.8**		**25.6**

Key: G.gazelle – Grant's gazelle; T.gazelle – Thomson's gazelle

Table 3.6: Dung pile counts of alternative host species in baboon group's habitat ranges at 100% MCP.

Dung counts at 100% MCP in wet and dry seasons													
	Weaver		**Viola**		**Snap**		**Narasha**		**Mica**		**Hokey**		Mean
Hosts	**Dry**	**Wet**	**Dry**	**Wet**	**Dry**	**Wet**	**Dry**	**Wet**	**Dry**	**Wet**	**Dry**	**Wet**	
Cattle	266	176	312	161	419	294	211	212	293	243	284	344	**267.9**
Goat	33	31	43	65	148	143	65	143	103	145	103	216	**103.2**
G.gazelle	83	151	85	70	79	117	82	59	72	52	47	118	**84.6**
Impala	12	2	12	0	1	14	0	0	0	0	1	14	**4.6**
Sheep	63	23	42	37	127	87	52	63	72	78	114	131	**74.1**
T.gazelle	14	56	12	16	19	62	5	3	16	1	20	63	**23.9**
Wildebeest	131	250	107	134	96	317	15	9	17	5	50	118	**104.1**
Seasons mean	86	94.4	87.6	69	127	147	61.4	69.8	81.9	74.9	88.4	143.4	
Group mean	**90.2**		**78.3**		**137**		**65.6**		**78.4**		**115.9**		**94.2**

Key: G.gazelle – Grant's gazelle; T.gazelle – Thomson's gazelle

3.3.2.2 Frequency of alternative hosts sightings as an index of overlap

Sighting points of alternative species within home ranges of baboon groups demonstrated how the different species of alternative hosts were spatially scattered in the baboon ranges (Figure 3.8 and 3.9). The frequency of sighting alternative host species in baboon home ranges was used to infer degree of overlap with baboons. Therefore, the degree of overlap across baboon social groups was found to be variable at both 50% MCP and 100% MCP of the habitat ranges and specifically in the wet seasons (Table 3.7 and 3.8).

The degree of overlap (mean frequency sightings) in 100% MCP of the baboon range was larger (8.8) compared to the overlap in 50% MCP, which was 4.5 (Table 3.7 and 3.8). At 100% MCP of the habitat range (Table 3.7), Weaver was the baboon group with the highest overlap (mean frequency sighting at 16.6) while Narasha had the least overlap (mean frequency sighting at 2). At 50% MCP habitat range (Table 3.8), Weaver was the group with the highest overlap (frequency sighting at 8.4), while Narasha remains as the group with least overlap (frequency sighting at 0.5).

The alternative host species that had the highest degree of overlap with baboons at 100% MCP (Table 3.7) was Grant's gazelle (mean frequency sightings at 17.3) followed by Thomson's gazelle at 16.9, whereas at 50% MCP (Table 3.8), Thomson's gazelles had the highest degree of overlap with baboons (mean frequency sighting at 9.5) followed by Grant's gazelle at 8.5. Sheep was the species that consistently had the least degree of overlap with baboons at both 100% MCP and 50% MCP (Table 3.7 and 3.8).

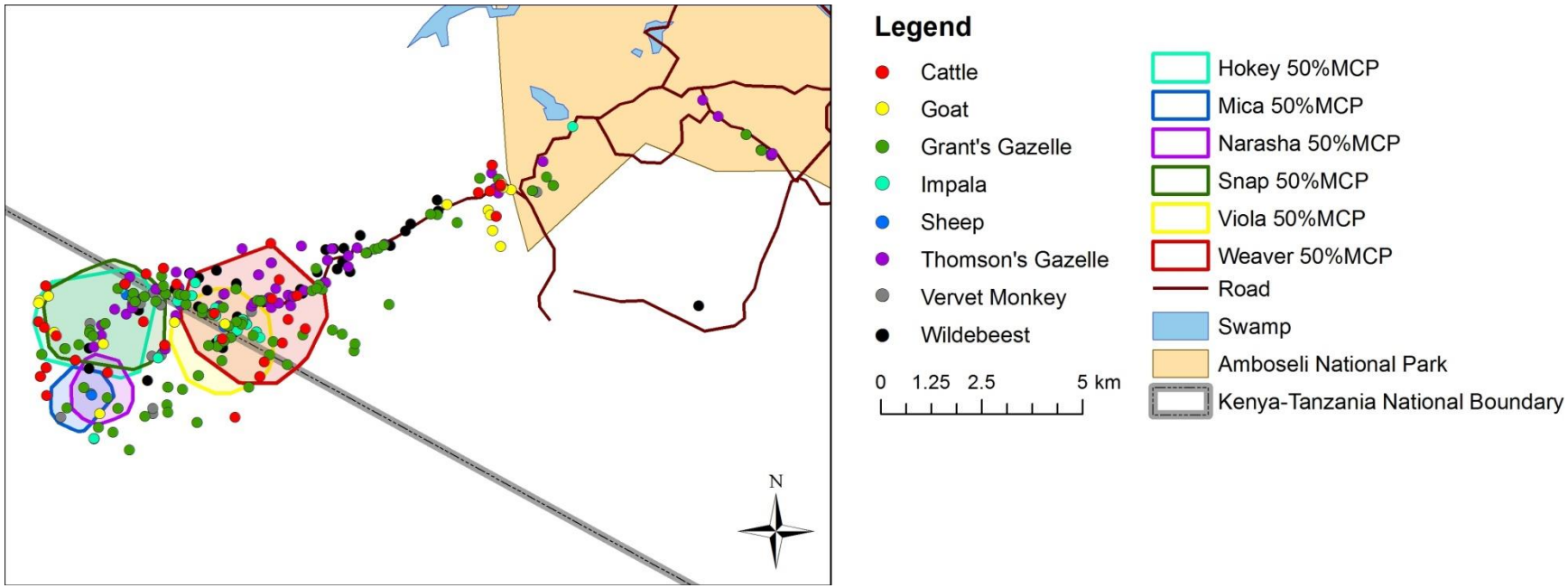


Figure 3.8: Map of the Amboseli ecosystem showing overlapping home ranges of baboon groups at 50% MCP and sighting points of alternative host species. Colored polygons are the home ranges of the six baboon groups. Colored dots show the sights where other alternative host species were observed.

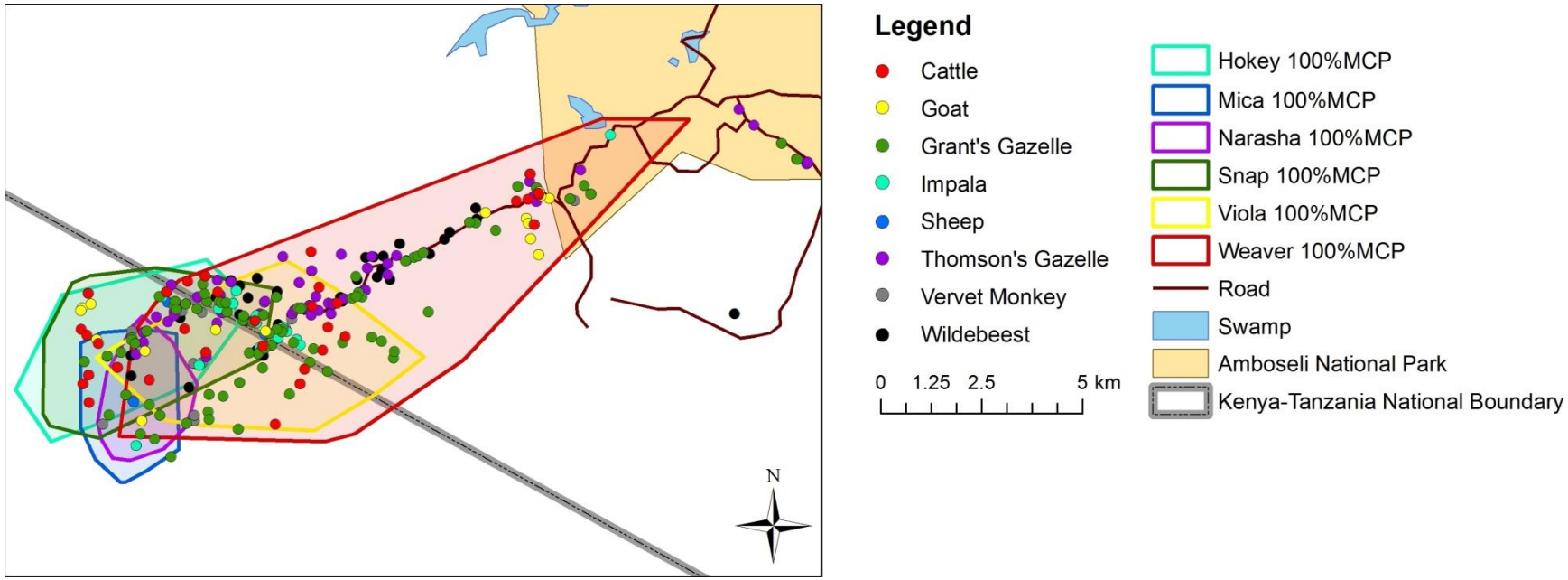


Figure 3.9: Map of the Amboseli ecosystem showing overlapping home ranges of baboon groups at 100% MCP and sighting points of alternative host species. Colored polygons are the home ranges of the six baboon groups. Colored dots show the sights where other alternative host species were observed.

Table 3.7: Frequency sightings of alternative host species in baboon group's habitat range at 100% MCP.

Host sightings in baboon groups during wet and dry seasons													
Hosts	**Weaver**		**Viola**		**Snap**		**Narasha**		**Mica**		**Hokey**		**Mean**
	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	
Cattle	6	12	7	8	9	11	2	0	3	1	5	5	**5.8**
Goat	3	12	5	3	9	6	2	2	4	1	6	3	**4.7**
G.gazelle	13	57	13	39	10	47	1	9	4	3	7	5	**17.3**
Impala	6	13	6	11	3	14	0	2	0	1	6	7	**5.8**
Sheep	3	12	3	3	4	7	1	2	2	1	3	1	**3.5**
T.gazelle	18	56	15	22	17	48	2	1	6	1	10	7	**16.9**
V.monkey	0	18	7	14	7	21	0	5	0	4	3	15	**7.8**
Wildebeest	3	34	3	14	1	33	1	2	0	2	2	11	**8.8**
Seasonal mean	6.5	26.75	7.37	14.25	7.5	23.37	1.125	2.87	2.37	1.75	5.25	6.75	
Group mean	**16.6**		**10.8**		**15.4**		**2**		**2.1**		**6**		**8.8**

Key: G.gazelle – Grant's gazelle; T.gazelle – Thomson's gazelle; V.monkey – vervet monkey

Table 3.8: Frequency sightings of alternative host species in baboon group's habitat range at 50% MCP.

Host sightings in baboon groups during wet and dry seasons													
Hosts	**Weaver**		**Viola**		**Snap**		**Narasha**		**Mica**		**Hokey**		**Mean**
	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	
Cattle	6	6	7	2	9	1	0	0	3	0	5	0	**3.3**
Goat	3	11	5	1	9	0	1	1	4	1	6	1	**3.6**
G.gazelle	13	21	13	15	10	10	0	1	4	3	7	5	**8.5**
Impala	6	0	6	7	3	2	0	0	0	1	6	0	**2.6**
Sheep	3	11	3	1	4	0	1	1	2	1	3	1	**2.6**
T.gazelle	18	23	15	1	17	14	2	0	6	1	10	7	**9.5**
V.monkey	0	2	7	6	7	10	0	0	0	2	3	5	**3.5**
Wildebeest	3	9	3	4	3	5	1	0	1	0	1	1	**2.6**
Seasonal mean	6.5	10.37	7.37	4.62	7.75	5.25	0.62	0.37	2.5	1.12	5.12	2.5	
Group mean	**8.4**		**6**		**6.5**		**0.5**		**1.8**		**3.8**		**4.5**

Key: G.gazelle – Grant's gazelle; T.gazelle – Thomson's gazelle; V.monkey – vervet monkey

3.4 Discussion

3.4.1 Degree of habitat overlap among baboons social groups

The present study showed that most of the habitats of baboon groups were overlapped (Figure 3.6 and 3.7), which is not uncommon in baboon society (Aldrich-Blake *et al*., 1971; Smuts, 1985; Cawthon, 2006). However, the degree of overlap in the current study was considerably high (Table 3.1 and 3.2) perhaps influenced by the poor and degraded environment in the Amboseli ecosystem (Altmann *et al*., 2002). Food (pasture and prey) and water are key habitat resources that are needed by animals (Darmon *et al*., 2012), however, they are subject to fluctuate in quantity and quality. This means when a habitat has reduced quantity and quality of water and food then it is considered poor and vice versa.

In baboon social organization, home ranges are usually defined and aggressively defended by the alpha males, though intruding groups can only be tolerated under special circumstances (Aldrich-Blake *et al*., 1971). It has been documented that animals in poor habitats tend to have large overlapping habitats whereas those in rich habitats occupy small exclusive home ranges (South, 1999; McLoughlin *et al*., 2000; Pearce *et al*., 2013). This is because baboons in poor habitats are more likely to spend more time foraging, with limited time or energy to spend patrolling the home ranges and engaging in agonistic encounters (Pearce *et al*., 2013).

In the present study, home ranges at 100% MCP of habitat were highly overlapped in both wet and dry seasons (Tables 3.1 and 3.2). In contrast, at 50% MCP, the sizes of overlapped areas were variable and relatively lower (Table 3.3 and 3.4) which suggests

that within the core area of habitat, inter-group interactions are dynamic and mostly less tolerated.

The seasonal effect on degree of overlap revealed that at 50% MCP of habitat, which represents the core area of a social group, the degree of habitat overlap in the dry season (mean area of overlap 2.45 km^2) was much higher (Table 3.3) compared to the level (mean area of overlap 0.44 km^2) in the wet season (Table 3.4), an observation which was consistent with that of McLoughlin *et al*., (2000) and fundamentally displays the nature of inter-group relations at core habitat level. The change in degree of overlap between seasons especially at core habitat level suggests that the nature of inter-group relations in Amboseli baboon is dynamic and changes between seasons whereby inter-group interactions are more tolerated in the dry season and less in the wet season. This suggests that the nature of inter-group interactions among the Amboseli baboons is resource based, whereby other conspecific groups are tolerated to overlap when food and water are inadequate and vice versa. Such observation is not unique to Amboseli baboon population as Pearce *et al*., (2013) explains that unlike territoriality, which is a fixed species trait, home range is rather plastic and its patterns consistent with economics of defendability (Mitani and Rodman, 1979; Waser and Homewood, 1979). This means that baboons consider it worthless defending territories in poor habitats or during unproductive seasons compared to if productivity was high (Barton *et al*., 1992; South, 1999; McLoughlin *et al*., 2000).

Size of the habitat range for each baboon group varied between seasons and at different habitat levels. At 100% MCP, habitat size ranged from 5.9 km^2 in Narasha to 15.59 km^2 in Weaver with the entire population mean of 10.47 km^2 during the dry season (Table

3.1). The ranges for most of the groups increased in the wet season, with the largest still being in Weaver (57.61 km^2) and the least, Narasha (5.09 km^2) and the population mean range of 24.23 km^2 (Table 3.2). A similar pattern was observed at 50% MCP of the habitat (Tables 3.3 and 3.4). First, Weaver, being the largest social group (*n* = 117; 33%), maintained a large home range in both seasons, an observation explained by the notion that large social groups occupy and effectively defend larger territories (Chapman and Chapman, 2000; Hoffman and O'riain, 2012). Second, Wahungu, (2001) observed similar pattern of seasonal variation in home range size as well as daily-path length in the Olive baboon population in Tana River forest, Kenya, which was linked to seasonal variation in food availability. In Amboseli baboon population, seasonal change in foraging pattern, ranging behavior, daily activity patterns and diet composition has been documented (Alberts *et al*., 2005).

In summary, variations in the size of home ranges and degree of overlap across social groups apparently suggest that baboon population in Amboseli occupy highly overlapped smaller home ranges in the dry season compared to the sizes in the wet season, which are larger but minimally overlapped. This pattern likely depicts behavior of baboon populations in savannah grasslands, which is contrasted with forested habitats (Norton *et al*., 1987; Wahungu, 1998; 2001).

The mean home range size of 10.46 - 24.23 km^2 in the present study was comparable to home ranges of baboons that generally inhabit savanna grassland. For example, Harding, (1976) documented a home range size of 19.7 km^2 for Olive baboon population (*n* = 49) at Gilgil, Kenya, whereas Olive baboon population (*n* = 100) at Laikipia Plateau, Kenya had home range size of 43.8 km^2 (Barton *et al*., 1992). In contrast, size of home ranges of

baboons in forest ecosystems, such as Ishasa in Uganda and Gombe National Park in Tanzania, are relatively smaller (3.88 - 5.18 km²), which indicate rich habitat (Rowell, 1966). Moreover, Olive baboon population (*n* = 15 -24) that inhabited Bole valley, Ethiopia, had even smaller home ranges as low as 0.74 to 1.12 km², which could mean the habitat was much richer (Dunbar and Dunbar, 1974).

3.4.2 Degree of habitat overlap between baboons and alternative host species

3.4.2.1 Degree of habitat overlap based on dung pile counts

The degree of overlap between baboons and alternative host species based on dung pile counts was variable at both 50% MCP and 100% MCP of habitat levels (Table 3.5 and 3.6). This implies that the level of occupancy in the habitat was heterogeneous and that some baboon groups experienced greater intensity of disturbances by heterospecifics than others. According to Strum and Western, 1982, high density of heterospecifics, particulalrly the wild and domestic ungulates, negatively affects the population performance of baboon populations. Since, spatial distribution of animals is often influenced by spatial distribution of key resources, which include but not limited to water, food and safety (Zweifel-Schielly *et al*., 2009; Hopcraft *et al*., 2012; Bennit *et al*., 2014), the social groups that experienced higher habitat overlaps (Hokey and Weaver) were likely to be located in areas that have key resources needed by multiple hosts. Hokey and Weaver occupied areas that had natural spring water, which were the only water source in the area. In contrast, Narasha is the group that consistently had the least overlap at both 50% and 100% MCP habitat levels (Table 3.5 and 3.6), which ,may suggest that its habitat location is of minimal preference or inadequate resources for the heterospecifics.

Cattle were the host species that had highest overlap across baboon groups in both 50% and 100% MCP of habitat levels (Table 3.5 and 3.6). Explanation for such observation may be that, first, since dung count was used as an index of overlap, it is likely that cattle dung persists more compared to the dung pellets from other host taxa especially in such hot, dry and dusty environment of Amboseli ecosystem. Second, it may portray the extensive grazing nature of the pastoralist cattle. In contrast, impala was the host species that had least overlap with baboons (Table 3.5 and 3.6), suggesting its key resource in the area was clumped hence they were spatially segregated.

There was lack of seasonal pattern in the degree of habitat overlap between baboons and heterospecifics (Table 3.5 and 3.6), perhaps pointing out that even though seasons have effect on resource quality and quantity, the response to such seasonal changes in terms of spatial habitat selection and preference by different host taxa may not be homogenous.

3.4.2.2 Degree of habitat overlap based on frequency of sightings

Degree of habitat overlap between baboon and heterospecifics was variable across baboon groups and did not display seasonal pattern (Table 3.7 and 3.8). This implies that, first, some baboon groups had greater overlaps compared to others and second, seasonal habitat selection and habitat preference by the different sysmpatric hosts was not homogenous. According to Hopcraft *et al*., (2012), spatial distribution of herbivores in a habitat is strongly influenced by resource availability and risks of predation. This means that animal species whose niches overlap tend to be aggregated in a particular habitat patch, while the rest of the species with contrasting niches are segregated (Darmon *et al*., 2012). Since Weaver group had the highest degree of overlap at both 50% and 100% MCP of habitat (Table 3.7 and 3.8), this group experiences constant disturbance but also

implies that the patch occupied by Weaver is a resource rich area or quite safe against predation. In contrast, Narasha group consistently had the least degree of overlap both at 50% and 100% MCP of habitat (Table 3.7 and 3.8), however, less disturbance by conspecifics may also imply inhabiting resource poor patch of the habitat or bears high risks to predation. Medium to small bodied herbivores such as impala, Thomson's and Grant's gazelles avoid patches with high vegetation, woody cover or thickets, as a strategy against predation (Hopcraft *et al*., 2012).

Although humans influenced the movement of domestic ungulates, the degree of overlap between baboons and domestic ungulates did not show explicit pattern that can be associated with humans. Among the alternative sympatric animals, sheep was the species that had the least degree of overlap with baboons at 100% MCP of habitat (Table 3.7) while at 50% MCP of habitat, in addition to sheep, impala and wildebeest were also least overlapped (Table 3.8). It is most likely that the distribution of resource needs for these host species are clumped hence their occurrence in the habitat was aslo clumped. For example, sheep and wildebeest are ground level grazers in open fields and such habitat characteristics may be patchy. Moreover, sheep, impala and wildebeests are highly gregarious animals hence their spatial occurrence in the habitat is likely to be segregated. The resident wildebeest populations tends to occupy particular ranges throughout the year, dispersed in single territorial and small segregated herds (Estes, 1991). In contrast, Gazelles, which dispersed evenly in the Amboseli habitat (Figure 3.8 and 3.9), were the host species that displayed greater overlap with baboons both at 100% and 50% MCP of habitat levels (Table 3.7 and 3.8).

In summary, inter-group estimation of degree of overlap revealed that at maximum habitat ranges, baboon groups were highly overlapped. However, the core area of habitat was critical in defining co-occurrence within species social groups. This is because at 50% MCP, each social group overlapped with an average of at least one other social group and the overlap was less tolerated because degree of overlap (mean percent overlap) was lower (Table 3.3 and 3.4) compared to that of 100% MCP (Table 3.1 and 3.2). Moreover, at 50% MCP, the seasonal effect on overlap was revealed whereby during dry season inter-group overlap was more tolerated compared to the wet season.

The degree of habitat overlap between baboons and sympatric host species was revealed by both indices of animal presence whereby degree of overlap was variable across baboon groups. In terms of seasonal effect, both indices were in concordance that the degree of habitat overlap between baboons and the alternative sympatric host species lacked clear seasonal pattern. Furthermore, irrespective of 50% or 100% habitat levels, Weaver was the group that had high overlap with heterospecifics while Narasha had the least degree of overlap. This means that Weaver group experienced constant intrusion and disturbance even at its core habitat area, while Narasha group experienced fewer disturbances throughout its habitat range.

CHAPTER FOUR: HELMINTH PREVALENCE, ABUNDANCE AND SPECIES RICHNESS IN THE SYMPATRIC HOSTS IN AMBOSELI ECOSYSTEM, KENYA.

4.1 Introduction

Gastrointestinal helminths are ubiquitous endoparasites that may be considered integral part of host's internal flora. Helminths are transmitted through ingestion of infective stages in the environment. High host diversity and density is predicted to result in high pasture contamination with helminths infective stages, which eventually leads to high infection burden. The influence of host density on helminth infection levels has been widely studied (Anderson and May, 1978). In contrast, the effect of host ecological community on infection level has been rarely studied (Ezenwa, 2003; Woolhouse *et al*., 2001; Johnson *et al*., 2013). It is expected that as diverse hosts co-occur in a defined habitat patch they contribute to the helminth community in terms of both abundance and diversity.

This study sought to determine the effect of diverse host co-occurrence (habitat overlap) on infection levels of helminths. The diversity and sympatry of hosts in Amboseli ecosystem presented a suitable natural model to determine the effect of habitat overlap between baboons, vervet monkeys and ungulates on helminths prevalence, diversity and abundance.

4.2 Materials and Methods

4.2.1 Study design

Sampling was by cross-sectional method whereby freshly voided dung/pellets of encountered animals were collected at one point in time. The prevailing condition during three consecutive months before sampling described the season of sampling. For instance, wet season sampling was carried out in January/February, 2012 because October-December, 2012 received rain (Figure 3.2). As such, transmission of helminths that occurred before the rains begun is supposed to be within the prevailing dry conditions. On the other hand, dry season sampling was carried out in October, 2012 because there was no rainfall in July-September, 2012 (Figure 3.2). Nevertheless transmission of helminths is subject to pre-patent periods.

4.2.2 Sample size

The populations to be sampled were baboons and 'alternative hosts'. The baboon population is structured into six social groups, whereas 'alternative hosts' was a group of 8 different mammalian host taxa. The population size of 'alternative hosts',was regarded as 'one population', which infinite hence the sample size was determined using normal approximation to the binomial distribution according to the formula described by Martin *et al*., (1987).

$$n = \frac{Z^2(P)(1-P)}{d^2}$$

Where:

n = Sample size (infinite): Z = Z value (Confidence level, 95%)

P = prevalence (p = 0.5): d = precision (d = 0.05)

n = 384

The determined sample size (n = 384) was the minimum total number of the required samples from the 'alternative hosts'. Since there were eight different species that encompass 'alternative hosts', the total sample size was divided by eight (384/ 8 = 48), hence 48 samples were collected for each host taxa per season.

For baboons, since the actual population was known and small (N =358), a correction factor was used to adjust downward the sample size (n) obtained from infinite population (Martin *et al*., 1987). Therefore the adjusted sample size (n') was calculated as follows:

$$n' = \frac{1}{1/n + 1/N}$$

Where: n' = adjusted sample size

n = sample size (384)

N = population of baboon (358)

n' = 185

Since each social group varied in numbers, their sample size also varied in proportion of each group size (Table 4.1).

Table 4.1: Sample size for faecal material from baboon social groups

Baboon groups	Group size	Proportion of population %	Dry Season samples	Wet season samples	Total
Weaver	117	33	60	60	120
Hokey	72	20	37	37	74
Viola	64	18	33	33	66
Narasha	43	12	22	22	44
Mica	34	9	18	18	36
Snap	28	8	15	15	30
Total	**358**	**100**	**185**	**185**	**370**

4.2.3 Fecal sample collection

Wildlife and livestock herds were tracked by vehicle and observed for fecal voidance. Upon defecation sampling was carried out which, involved scooping approximately 1gram of faecal material from different bolus of dung/pellet middens (Figure 4.1). Portions of dung or faecal pellets (antelopes) were scooped from its core, away from soil contact. The sub-samples of the dung from a single animal were pooled to about 5 grams in a plastic container that was pre-filled with 10% formalin, mixed and labeled. Samples for faecal culture were collected separately. Approximately 10 grams of faecal material from each animal species were collected in plastic containers and kept fresh without any

preservative added. The samples were placed in cool box and transported to the field laboratory. Samples for coproscopic analysis were maintained at room temperature both in the field and until analysis was complete at the Kenya Willdife Service Veterinary Laboratory in Nairobi. Samples for culture were immediately prepared in the field and were transported to Nairobi laboratory for completion of culture and larval isolation.

Figure 4.1: Faecal sample collection in Amboseli ecosystem, Kenya.

4.2.4 Coproscopic parasitological analyses

4.2.4.1 Sedimentation method

The method by Vanderwaal *et al*., (2014) was adopted in which fecal pellets were first crushed using a pestle to homogenize them within the sample container. Three grams of the fecal sample was mixed with 45 ml of tap water in a 50ml centrifuge tube, stirred and strained using a tea strainer. The filtrate was left to sediment for at least 10 minutes and then suspension gently decanted out. The sediment was re-suspended with 45 ml of water and left to stand for further 10 minutes. Re-suspension and decanting was repeated until the suspension was clear. 200µl of the sediment was pippeted onto a glass slide and

covered with cover slip (32 x 24 mm). Four slide preparations of the sediment of each fecal sample were examined under the microscope (Leica DM500, Leica microsystems, UK) at x100 magnification. Microimages were taken and dimensions of helminth ova measured using the LAZ EZ microimaging software version 2.0 (Leica microsystems, UK).

4.2.4.2 Floatation method

A method recommended by Gillespie (2006) was applied in principle but slightly modified in procedure. Sheather's solution was used as the floatation fluid instead of Sodium nitrate as recommended by Gillespie *et al*., (2006). Floatation fluid was prepared by mixing 454g of table sugar (Mumias Sugar co. Kenya) and 355 ml of distilled water. The mixture was heated over low heat while intermittently being stirred until sugar dissolved. The solution was left to cool before being used. Four grams of homogenized fecal samples that remained from the sedimentation method was weighed and mixed with 12 ml of tap water until it was slurry. The slurry was sieved through a tea strainer and the filtrate transferred into a 15 ml plastic centrfuge tube. If the filtrate was less than 14 ml, it was topped up with tap-water to 14 ml mark, the tube capped and centrifuged at 1500 rpm for 10 minutes. The supernatant was decanted out and floatation fluid (sugar solution) added up to half of the tube. The sediment was mixed thoroughly with the floatation fluid using a stirring stick. The tube was then filled to the top with more floatation fluid until it formed a slight bulging meniscus. A cover slip was gently placed on top of each tube ensuring the cover slip was centred well on top of the tube. These tubes capped with coverslips were centrifuged for 10 minutes at 1500 rpm. After, centrifugation, the cover slip was gently removed and placed on a glass slide. The glass

slide was examined under the microscope at x100 magnification for identification and measurement of at least 10 eggs of each helminth egg type. As such, egg count was based on absolute counts.

4.2.5 Data management

Individual samples that were positive were recorded in data sheets which were entered in Microsoft Excel sheet. Prevalence was determined as a percentage of positive samples from the total sample size for each host taxa. The number of helminth taxa in each host taxa was enumerated to generate the parasite species richness. Abundance was recorded as the absolute count of helminth eggs observed under each slide.

Chi-square was used to test whether prevalence in helminths differed 1) across hosts 2) between baboon social groups, 3) by season across all host species 4) by season across all baboon social groups and 5) between non-human primates, wild and domestic ungulates and between ungulates and non-human primates.

Dimensions (length and width) of individual helminths eggs were measured and entered in Excel sheet. The data was uploaded into R statistical software, which was used to generate box plots that demonstrate difference in dimensions between eggs of *Trichuris sp* and *Moniezia* sp. across hosts. Analysis of Variance (ANOVA) was used to test variation in *Trichuris* sp. eggs isolated from different hosts. Students't test was used to analyse difference in *Moniezia* sp. eggs isolated from different hosts.

The number of helminths eggs in each sample from particular host species was enumerated and recorded in Microsoft Excel sheet. Mean abundance (dry and wet season egg counts) for each host taxa was also calculated.

4.3 Results

4.3.1: Sedimentation parasitological method

4.3.1.1: Prevalence of helminths in all host species

Presence of any egg of a species of helminths in a sample was considered as helminths positive. The total number of helminths positive samples in each host was divided by the total number of samples for that host and multiplied by 100% to generate prevalence of heminth for each host taxa. The mean prevalence of helminths (combined prevalence in the wet and dry season) across host species is listed in Table 4.2. Prevalence of helminths was significantly different across all host species (χ^2 = 200.37, df = 8, p = 0.0001) and baboons had the least prevalence while Grant's gazelles had the highest prevalence (Table. 4.2).

Table 4.2: Mean prevalence by sedimentation and floatation techniques in different host species in Amboseli ecosystem, Kenya in both seasons

Host species	**Sedimentation technique**	**Floatation technique**	**Overall Prevalence (%)**
	Mean prevalence (%)	Mean prevalence (%)	
Grant's gazelle	97.9	98.9	98.4
Thomson's gazelle	75	100	87.5
Goat	73.9	96.8	85.3
Sheep	63.5	86.4	74.9
Impala	62.5	87.5	75
Vervet monkey	62.5	83.3	72.9

Cattle	56.2	94.7	75.4
Wildebeest	38.5	95.8	67.1
Baboon	31.9	77.3	54.6

All species, n = 48; Baboon, n =185

Figure 4.2 shows seasonal variation in helminth prevalence across host taxa. The host taxa showed a seasonal pattern of infection. Except for baboon and Grant's gazelle, all the alternative hosts had higher prevalence of helminths in the wet compared to dry season (Figure 4.2). In contrast, baboon had higher prevalence (42.2%) during the dry season compared to the wet season (21.6%). Similarly, Grant's gazelle had 100% prevalence in the dry season and 98.5% in the wet season. However, in all the host species, prevalence of helminths was significantly different (χ^2 = 23.87, df = 1, p = 0.0001) between wet and dry season (Figure 4.2).

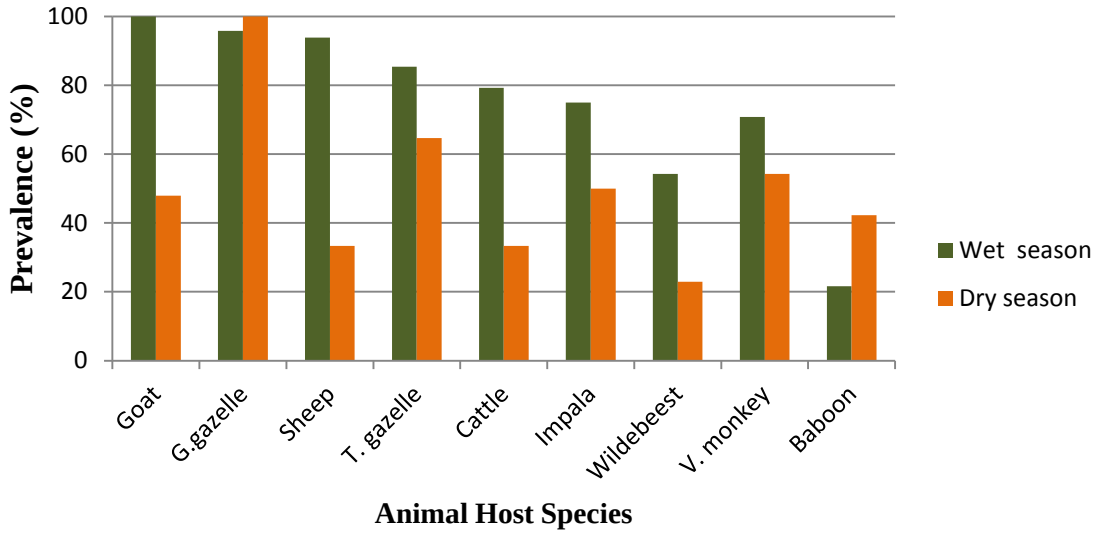


Figure 4.2: Seasonal variation in prevalence of helminths across the sympatric host species in Amboseli ecosystem, Kenya. Key: G – Grant's; T – Thomson's; V – Vervet.

The prevalence of different helminth genera across host species in the sympatric community at Amboseli are listed in Table 4.3. There are many nematodes that produce strongyle type eggs, hence hereafter all such egg types represented the nematodes that was collectively refered to as the 'strongylids'. Goat and sheep were infected by strongylids, *Strongyloides* sp., *Spirurid sp*-morphotype A (Nematodes) and *Moniezia* spp. (Cestode). Specifically, Goat was infected with *Moniezia benedini* while sheep had both *M. benedini* and *M. expansa* (Table 4.3). Cattle were infected by stongylids, *Spirurid sp*-morphotype A (Nematodes), *Paramphistomum* sp. and *Fasciola* spp. (Trematode). Wildebeests were infected with strongylid, *Spirurid* sp-morphotype A (Nematode) and *Fasciola sp.* (Trematode). Thomson's gazelles were infected with strongylids, *Spirurid* sp-morphotype A., *Trichuris* sp. (Nematode) and *Fasciola* sp. (Trematode). Grant's gazelle had strongylids, *Strongyloides* sp. and *Trichuris* sp. (Nematode). Impala was only infected with strongylids. Both vervet monkey and baboon were infected with strongylids, *Trichuris* sp., *Enterobius* sp., *Primasubulura* sp. and *Spirurina* sp., (Nematode). Additionally, baboon was infected with *Streptopharagus* sp. (Nematode).

Across the different types of helminths identified by sedimentation technique, strongylids were common across host taxa (Table 4.3). Moreover, strongylids was the highest in prevalence across hosts except in vervet monkey and baboon in which *Trichuris* sp. was the dominant helminth (Table 4.3). Among the ungulates, Grant's gazelle had the highest strongylid prevalence (95.8%) while the least prevalence (39.6%) was in Wildebeest (Table 4.3). Across host taxa, baboon had the lowest (1%) strongylid prevalence (Table 4.3).

Table 4.3: Prevalence (%) of different types of helminths eggs across wild and domestic animals in Amboseli ecosystem, Kenya

Helminth types	**Animal hosts**								
	Goats	Sheep	Cattle	Wildebeests	T.gazelles	G.gazelles	Impala	V.monkeys	Baboons
Strongylids	71.8	64.6	57.3	39.6	60.4	95.8	70.8	6.2	1
Moniezia benedini	2.1	0	0	0	0	0	0	0	0
Moniezia expansa	2.1	2.1	0	0	0	0	0	0	0
Strongyloides	5.2	1.1	0	0	0	2.1	0	0	0
Spirurid- morphotype A	1.1	4.2	5.2	2.1	3.1	0	0	0	0
Fasciola hepatica	0	0	3.2	2.1	2.1	0	0	0	0
Trichuris	0	0	0	0	35.4	49.9	0	53.2	37.2
Enterobius	0	0	0	0	0	0	0	5.2	0.5
Primasubulura	0	0	0	0	0	0	0	29.1	24.3
Fasciola gigantica	0	0	3.2	0	0	0	0	0	0
Paramphistomum	0	0	4.2	0	0	0	0	0	0
Spirurina	0	0	0	0	0	0	0	10.4	2.1
Streptopharagus	0	0	0	0	0	0	0	0	1

All species, n = 48; Baboon, n = 185. Key: T - Thomson's; G – Grant's: V - Vervet

4.3.1.2: Prevalence of helminths across baboon social groups

Prevalence of helminths across the baboon social groups was significantly different (χ^2 = 22.43, df = 5, p = 0.0001). Seasonal pattern was also statistically significant (Figure 4.3) whereby prevalence of helminths in dry season was higher (18.26, df = 5, p = 0.003) compared to wet season. During the dry season, Narasha group had the highest prevalence followed by Snap, Hokey, Viola, Mica and the least being Weaver (Figure 4.3). In the wet season, Snap had the highest prevalence followed by Narasha, Mica, Viola, Weaver and least being Hokey (Figure 4.3).

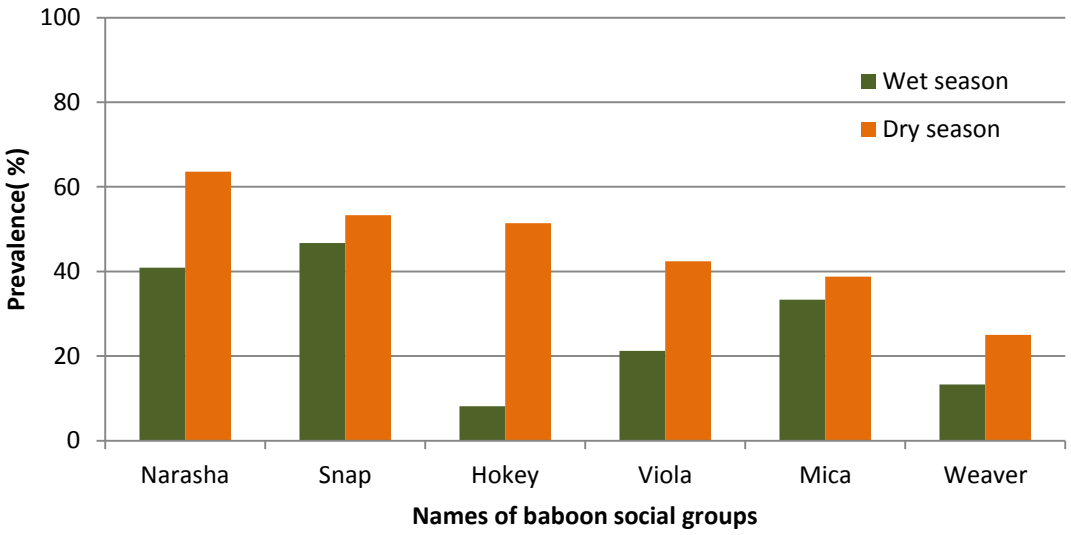


Figure 4.3: Seasonal variation in prevalence of helminths across baboon groups in Amboseli ecosystem, Kenya

Mean prevalence of helminths indicated that the most prevalent helminths in baboon were *Trichuris sp.* followed by *Primasubulura sp.* (Table 4.4). Prevalence of both *Trichuris sp.* and *Primasubulura sp.* increased in the dry season while that of strongylid remained the same in both wet and dry season (Table 4.4). Other helminths species,

Spirurina sp., *Enterobius* sp. and *Streptopharagus* sp. were observed only in the wet season (Table 4.4).

Table 4.4: Prevalence of helminths in baboons by sedimentation method

Wet season				Dry season		
Helminth species	***n***	**+ve**	**Prevalence (%)**	**+ve**	**Prevalence (%)**	**Mean prevalence (%)**
Trichuris sp	185	20	10.8	49	26.4	37.2
Primasubulura sp	185	21	11.3	24	12.9	24.3
Strongylids	185	2	1	2	1	1
Spirurina sp	185	4	2.1	0	0	2.1
Enterobius sp	185	1	0.5	0	0	0.5
Streptopharagus sp	185	2	1	0	0	1

Key: +ve – number of samples positive with a helminth

4.3.1.3: Prevalence of helminths between ungulate and non-human primate hosts

Since the sympatric host taxa could be categorized into broadly related taxonomic groups, prevalence of helminths was compared between three groups; non-human primates, domestic- and wild-ungulates (Figure 4.4). In non-human primates, which included vervet monkey and baboon, prevalence of helminths was significantly higher (χ^2 = 30.29, df = 1, p = 0.0001) in vervet monkey (62.5%) compared to prevalence of 31.9% in baboon (Table 4.2). Prevalence of helminths in wild ungulate hosts' group (68.5%) was not significantly different (χ^2 = 1.13, df = 1, p = 0.287) compared to that of domestic

ungulates group (64.6%). However, the prevalence of helminths in all ungulates (wild and domestic hosts) 66.8% (449/672) was significantly higher (χ^2 = 92.63, df = 2, p = 0.0001) compared to prevalence in non-human primates group [38.2% (179/469)]. Seasonaly, prevalence of helminths across host groups was significantly different in the wet season (χ^2 = 22.8, df = 1, p = 0.0001) compared to the dry season (Figure 4.4.), though seasonal infection pattern was not identical. For instance, in non-human primates prevalence of helminths in the wet season (49%) was lower compared to prevalence (49.9%) in the dry season (Figure 4.4). In contrast, prevalence of helminths in domestic ungulates (91%) and wild ungulates (77.6%) were higher in the wet season compared to prevalence in the dry season 38.2% and 59.4%, respectively.

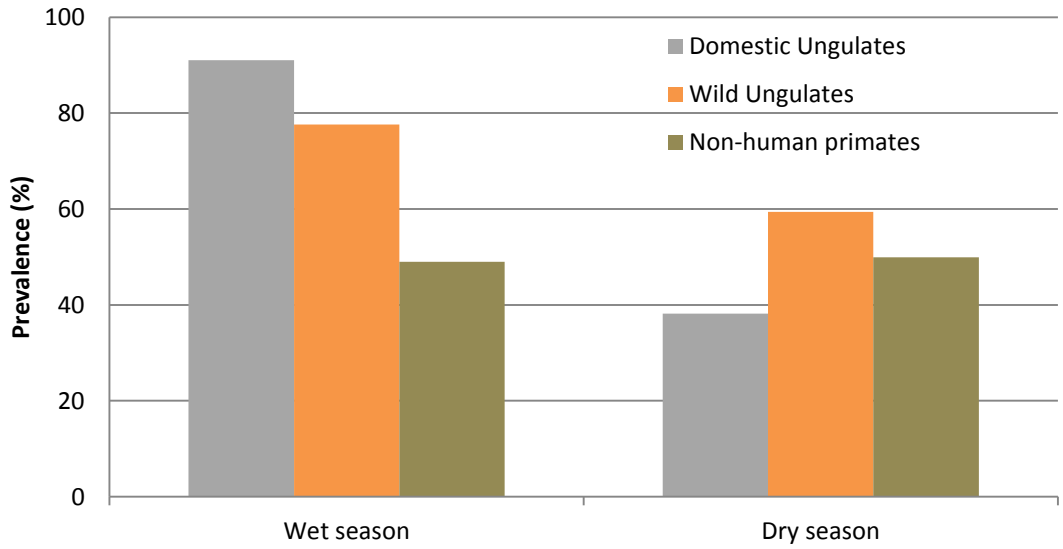


Figure 4.4: Seasonal prevalence of helminths across host taxonomic groups: Domestic ungulates i.e cattle, sheep and goats; Wild ungulates i.e impala, wildebeest, Grant's gazelle and Thomson's gazelle and non-human primates i.e vervet monkeys and baboons.

4.3.2: Floatation method

4.3.2.1: Prevalence of helminths in all host species

The mean prevalence of helminths across host species is listed in Table 4.5. The prevalence of helminths was high in all host taxa ranging from a low of 80% in vervet monkey to 100% in Thomson's gazelles. Prevalence of helminths across host taxa was significantly different when baboon was included in the host taxa (χ^2 = 54.505, df = 8, p<0.0001, Table 4.5) and when baboon was excluded (χ^2 = 48.342, df = 7, p<0.0001, Table 4.6).

Table 4.5: Variation in helminths prevalence across host species including baboons in Amboseli ecosystem, Kenya

Host Species	Number of animals		Total	Prevalence (%)
	Helmiths absent	Helminths present		
Thompson's gazelle	0	96	96	100.00
Grant's gazelle	1	95	96	98.96
Goat	3	93	96	96.88
Wildebeest	4	92	96	95.83
Cattle	5	91	96	94.79
Impala	12	84	96	87.50
Sheep	13	83	96	86.46
Baboon	57	313	370	84.59
Vervet monkey	19	77	96	80.21
Mean				89.98

Table 4.6: Variation in helminths prevalence across host species excluding baboons in Amboseli ecosystem, Kenya

Species	Number of animals		Total	Prevalence (%)
	Helminths absent	Helminths present		
Thomson's gazelle	0	96	96	100.00
Grant's gazelle	1	95	96	98.96
Goat	3	93	96	96.88
Wildebeest	4	92	96	95.83
Cattle	5	91	96	94.79
Impala	12	84	96	87.50
Sheep	13	83	96	86.46
Vervet monkey	19	77	96	80.21
Mean				92.58

In contrast, a different pattern was observed in that when baboon was included in the host list, prevalence of helminths did not differ significantly between wet and dry seasons (χ^2 =1.180, df = 1, p = 0.277), across host taxa (Table 4.7). When baboon was excluded in the host list (Table 4.8), prevalence between wet and dry season was significantly different (χ^2 = 8.357, df = 1, p = 0.004) across host taxa. Overall, prevalence of helminths in all species was higher in the wet season compared to the dry season (Table 4.7 and 4.8).

Table 4.7: Seasonal prevalence of helminths in all host species (including baboons) in Amboseli ecosystem, Kenya

Season	Number of animals		Total	Prevalence of helminths (%)
	Helminths absent	Helminths present		
Dry	63	506	569	88.93
Wet	51	518	569	91.04
Mean	57	512	569	89.98

Table 4.8: Seasonal prevalence of helminths in all host species (excluding baboons) in Amboseli ecosystem, Kenya

Season	Number of animals		Total	Prevalence of helminths (%)
	Helminths absent	Helminths present		
Dry	39	345	384	89.84
Wet	18	366	384	95.31
Mean	28.5	355.5	384	92.58

4.3.2.2: Prevalence of helminths across baboon social groups

Prevalence of helminths across baboon groups is shown in Table 4.9. The prevalence across baboon groups were significantly different (χ^2 = 27.754, df = 4, p<0.0001). Narasha and Snap were combined in order to satisfy the theoretical expectations (expected values were <5) for a chi-square test (Table 4.9).

Table 4.9: Prevalence of helminths across baboon social groups in Amboseli ecosystem, Kenya

Social group	Number of animals		Total	Prevalence (%)
	Helminths absent	Helminths present		
Hokey	12	62	74	83.78
Mica	16	20	36	55.56
Snap & Narasha	7	67	74	90.54
Viola	6	60	66	90.91
Weaver	16	104	120	86.67
Total	57	313	370	84.59

The effect of season on prevalence was however not evident since across baboon groups prevalence in the wet and dry seasons did not differ significantly ($\chi^2 = 1.680$, df = 1, $p = 0.195$, Table 4.10).

Table 4.10: Seasonal prevalence of helminths in baboon in Amboseli ecosystem, Kenya

Season	Number of animals		Total	Prevalence (%)
	Helminths absent	Helminths present		
Dry	24	161	185	87.03
Wet	33	152	185	82.16
Total	57	313	370	84.59

4.3.3: Abundance of helminths

4.3.3.1 Abundance of helminths eggs across all sympatric hosts

Abundance refers to the mean number of parasites or propagules in an infected host (Altizer *et al*., (2003). Abundance is usually determined by eggs per gram (epg), however, in the present study, abundance was determined by the absolute count of helminth eggs in each host taxa as described by Gillespie, (2006). Absolute count of helminths is adavantagious for use in non-human primates dwelling in dry regions where heminth prevalence and burden is likely to be low. The mean abundance (combined wet and dry season) in decreasing order was highest in vervet monkey (119.7 eggs) followed

by goat (115.7 eggs), Grant's gazelle (91.2 eggs), Thomson's gazelle (78.5 eggs), baboon (56.4 eggs), sheep (56.2 eggs), cattle (16.8 eggs), wildebeest (13.1 eggs) and lowest in impala (10.6 eggs).

Figure 4.5 shows seasonal variation in mean abundance of helminths in all host species. Abundance was higher in the wet season for 5 out of 9 hosts. Specifically, Grant's gazelles, sheep, goats Thomson's gazelles and impala had a higher abundance of helminths in wet season compared to dry season. In contrast, vervet monkey, baboon, cattle and wildebeest showed a higher dry season pattern in abundance of helminths.

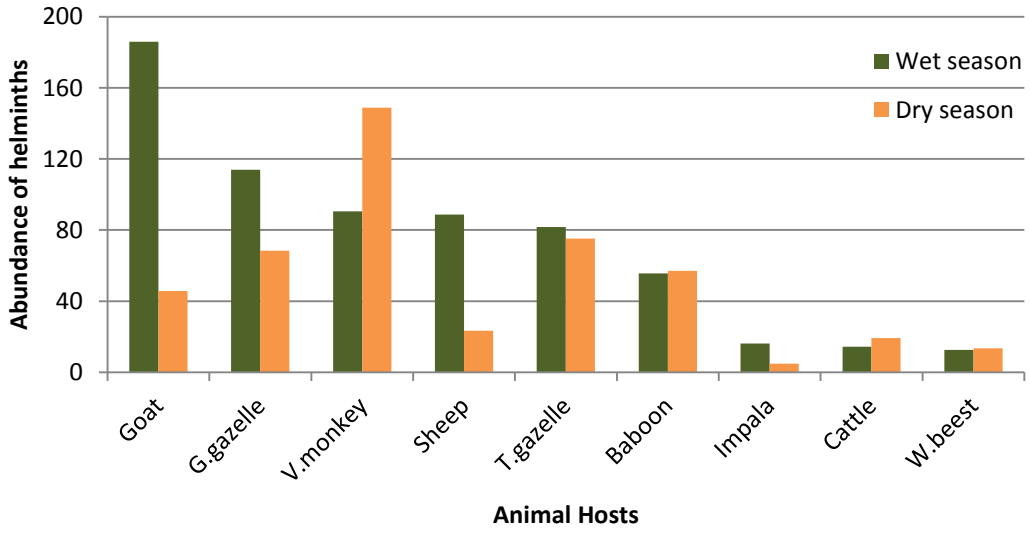


Figure 4.5: Seasonal abundance of helminths eggs across host taxa in Amboseli ecosystem, Kenya. Key – G.gazelle (Grant's gazelle); T.gazelle (Thomson's gazelle); W.beest (Wildebeest).

4.3.3.2 Abundance of helminths across baboon social groups

Figure 4.6 shows mean abundance of helminths eggs across baboon social groups. The mean abundance in decreasing order (wet and dry season counts) was highest in Narasha

(126.9), followed by Viola (119.1), Weaver (36.9), Hokey (28.3), Mica (14.5) and lowest in Snap (12.8). Mica and Narasha had higher abundance in the dry season compared to the wet season whereas in contrast, Viola and Weaver had higher abundance in the wet season (Figure 4.6). Hokey and Snap groups had abundance which was more or less identical (Figure 4.6).

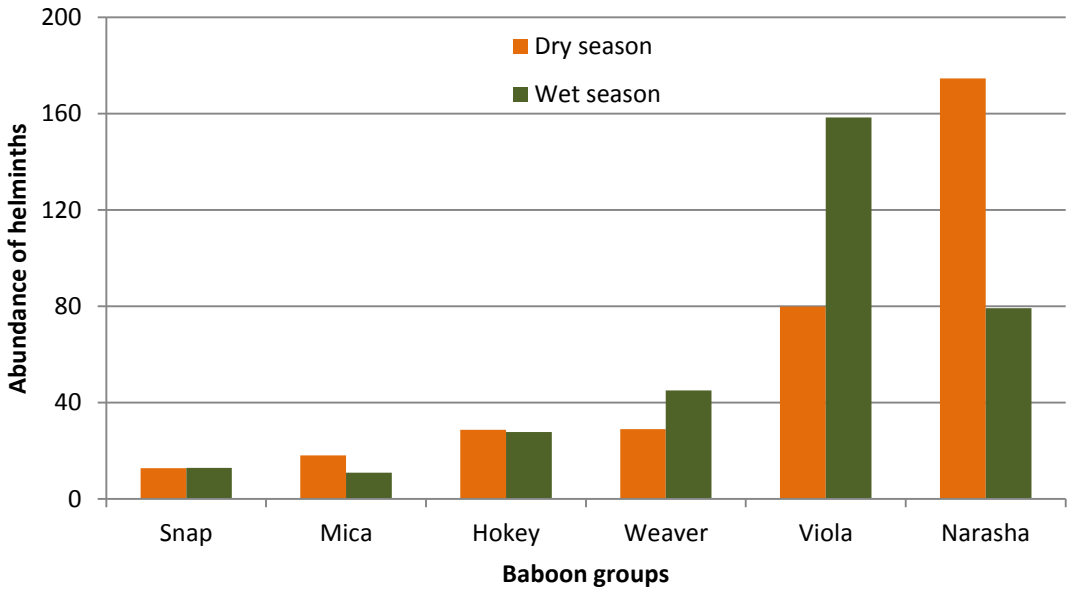


Figure 4.6: Seasonal differences in abundance of helminths across baboons groups in Amboseli ecosystem, Kenya.

4.3.4 Helminth species richness (HSR)

Table 4.11 shows the various species of helminths eggs identified using both the floatation and sedimentation techniques. In the entire host community, helminth species richness (HSR) ranged between two helminths to eight per host, with mean HSR of 5.1 ± 1.9. Except for Thomson's gazelle, Grant's gazelle and Impala all other host species had HSR above the mean HSR (>5) of the community (Table 4.11). Specifically, impala was

the host that had the least HSR by harboring only strongylids and *Trichuris sp.* Cattle and baboon were the two hosts with the highest HSR of seven and eight, respectively, (Table 4.11). Domestic ungulate host group had mean HSR (6.3 ± 0.58) more or less similar to that of the non-human primate host group (6.5 ± 2.12). Wild ungulate host group had the lowest mean HSR (3.5 ± 1.29).

Table 4.11: Types of helminths identified from animals in Amboseli ecosystem by both sedimentation and floatation methods. Key: "√" - presence of helminth eggs; "x" - absence of helminth eggs. OTU – operational taxonomic unit based on egg size

Helminth types	**Animal Host Species**									
	Goat	Sheep	Cattle	Wildebeest	T.gazelle	G.gazelle	Impala	V.monkey	Baboon	**Total**
Strongylids	√	√	√	√	√	√	√	√	√	**9**
Moniezia expansa	√	√	x	x	x	x	x	x	x	**2**
M. benedini (OTU-a)	x	x	√	√	x	x	x	x	x	**2**
M. benedini (OTU-b)	√	√	x	x	x	x	x	x	x	**2**
Trichuris (OTU-c)	√	√	√	√	√	√	√	x	x	**7**
Trichuris (OTU-d)	x	x	x	x	x	x	x	√	√	**2**
Strongyloides	√	√	x	x	x	√	x	x	√	**4**
Spirurid-morph A	√	√	√	√	√	x	x	x	x	**5**
Spirurid- morph B	x	x	x	x	x	x	x	x	√	**1**
Spirurina	x	x	x	x	x	x	x	√	√	**2**
Fasciola hepatica	x	x	√	√	√	x	x	x	x	**3**
Enterobius	x	x	x	x	x	x	x	√	√	**2**
Fasciola gigantica	x	x	√	x	x	x	x	x	x	**1**
Paramphistomum	x	x	√	x	x	x	x	x	x	**1**
Primasubulura	x	x	x	x	x	x	x	√	√	**2**
Streptopharagus	x	x	x	x	x	x	x	x	√	**1**
Helminth species richness (HSR)	6	6	7	5	4	3	2	5	8	**46**
Average HSR	6.3 ± 0.58			3.5 ± 1.29				6.5 ± 2.12		

A total of 16 types of helminths eggs were identified by both sedimentation and floatation fecal parasitological methods (Table 4.11). Out of these, eight were nematodes, which included *Enterobius* (Figure 4.7A), *Strongyloides* (Figure 4.7B), *Strongylids* (Figure 4.7C), *Primasubulura* (Figure 4.7D), *Trichuris* spp. (4.8A and D), unidentified species of *Spirurina* (Figure 4.9A), *Streptopharagus* (Figure 4.9B) and unidentified species of *Spirurids* (Figures 4.9C and D). *Moniezia* was the only cestode (Figures 4.8B and C). Trematodes included *Paramphistomum* spp. (Figure 4.9E) and *Fasciola* spp. (Figure 4.9F). The typical eggs of strongylid nematodes in the Order Strongylida (Figure 4.7C) are usually morphologically indistinguishable, thus were broadly categorized as 'strongylids', which is a non-taxonomical unit.

Figure 4.7: **Images of nematode eggs (A) *Enterobius* sp.; (B) *Strongyloides* sp.; (C) *Strongylid* type egg (D) *Primasubulura sp*. Magnification x400, Scale 50µm**

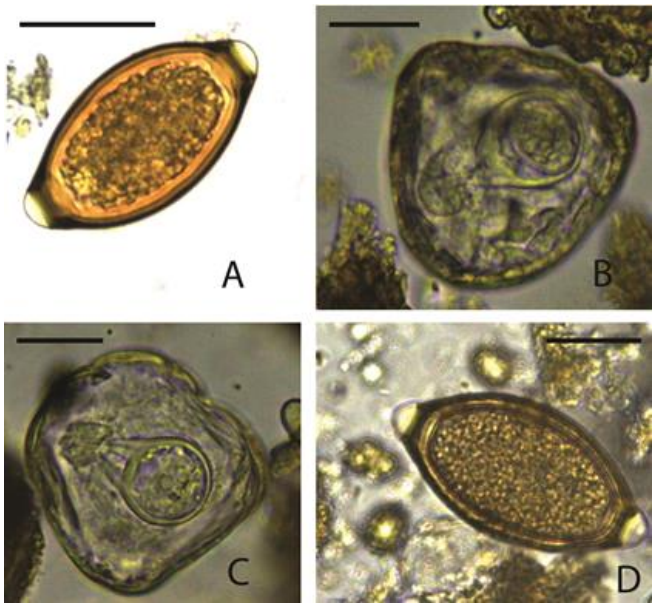


Figure 4.8: Images of helminth eggs (A) *Trichuris-OTU-d*; (B) *Moniezia expansa* (C) *Moniezia benedini* (D) *Trichuris-OTU-c*. Magnification x400, Scale 50µm

Figure 4.9: Images of helminth eggs (A) *Spirurina* sp. (B) *Streptopharagus* spp. (C) Unidentified *Spirurid*-morphotype A. (D) Unidentified *Spirurid*-morphotype B (E) *Paramphistomum* spp. (F) *Fasciola hepatica* Magnification x400; Scale: 50µm

Two different unidentified spirurids were found among the host community; *Spirurid*- morphotype A had a prominent double wall with the space in between the outer and the inner walls measuring mean diameter of 3.26 ± 0.5µm (Figure 4.9C). This *Spirurid* – morphotype A occurred in five different ungulate hosts i.e. goats, sheep, cattle, Thomson's gazelles and wildebeest (Table 4.11). *Spirurid*- morphotype B, had a distinct developing embryo (Figure 4.9D.) and occurred only in baboon.

There was a single incidence of an egg of an Acanthocephala (thorny-headed worm), which was identified as *Macracanthorhynchus hirudinaceus* - 97.22µm long by 61.04µm wide (Figure 4.10A) and a stomach fluke, *Protofasciola robusta* - 87.1µm long by 48.9µm wide (Figure 4.10B) in baboon samples.

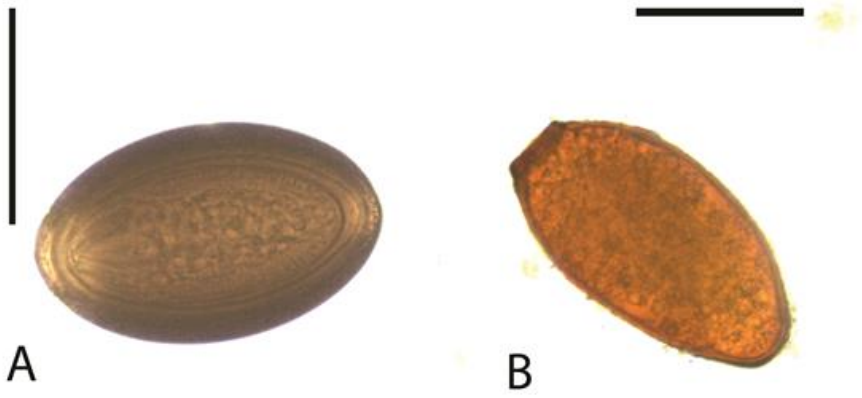


Figure 4.10: Images of spurious helminth eggs identified from baboons. (A) *Macracanthorhynchus hirudinaceus* (B) *Protofasciola robusta*. Magnification x400; Scale: 50µm.

Table 4.12 is a summary of the mean size and size range for all the helminths eggs. The size of eggs of *Trichuris sp.* and *Moniezia benedini* differed across host species and thus were categorized into operational taxonomic units (*OTU*) based on measured dimensions. The mean length of *Moniezia OTU-a*, which were isolated from cattle and wildebeest, were significantly larger ($t_{(17)}$ = -9.604, p<0.0001) compared to *Moniezia OTU-b* from sheep and goat (Figure 4.11). Similarly, mean width of *Moniezia OTU-a*, were also significantly larger ($t_{(17)}$ = -9.853, P<0.0001) compared to *Moniezia OTU-b* (Figure 4.11).

The mean length of *Trichuris OTU-c*, which were identified from six Antelopes, were significantly larger ($F_{(2)}$ = 210.8, P<0.0001) compared to *Trichuris OTU-d* from the two non-human primates (Figure 4.12). Similarly, the mean width of *Trichuris OTU-c* were also significantly larger ($F_{(2)}$ = 212.6, P<0.0001) compared to those of *Trichuris OTU-d.*

Table 4.12: The average sizes of different types of helminth eggs isolated from sympatric non-human primates, livestock and wild ungulates in Amboseli ecosystem.

Helminth	n	Mean size ± standard deviation		Range (max –min)	
		Length (µ)	Width (µ)	Length (µ)	Width (µ)
Moniezia benedini, OTU-a	8	92.2 ± 8.6	79.2 ± 1.8	105.1 - 82.7	82.6 – 76.5
Moniezia benedini, OTU-b	11	61.6 ± 3.3	58.4 ± 5.8	69.7 – 50.8	68.9 – 47.6
Moniezia expansa	14	72.9 ± 2.3	67.3 ± 5.8	78.8 -70.1	73.1 -48.1
Enterobius sp.	10	58.9 ± 1.1	28.8 ± 1.1	60.1 - 57.1	30.4 - 27.2
Primasubulura sp.	15	67.9 ± 3.4	55.1 ± 3.6	72.1 - 61.5	60.6 - 49.1
Strongyloides sp	6	53.8 ±1.9	32.5 ±1.1	56.4 – 50.5	34.2 - 31.1
Trichuris (OTU-c)	19	73.5 ± 2.8	36.1 ± 0.9	79.3 – 68.6	37.9 - 34.8
Trichuris (OTU-d)	31	60.1 ± 1.7	29.9 ± 1.2	63.7 - 56.5	32.1 - 27.3
Fasciola hepatica	11	148.2 ± 7.9	89.8 ± 4.8	158.9 -131.7	98.3 -81.5
Fasciola gigantica	8	160.4 ± 9	84.5 ± 6.1	172.1 -144.6	95.1 -75.2

Paramphistomum sp	4	160.6 ± 9.2	87 ± 5.9	166.1 -146.8	90.3 -78.1
Streptopharagus sp	2	40.1 ± 1.9	24.1 ± 7.4	41.4 -38.8	29.4 – 18.8
Spirurina sp.	16	55.9 ± 2.5	43.7 ± 2.1	59.8 – 50.5	47.3 - 40.9
Spirurid- morphotype A	15	62.5 ± 5.0	36.4 ± 3.6	69.9 -55.2	46.9 – 34.2
Spirurid-morphotype B	1	85	45	85	45

Key: n = sample size: max- maximum; min - minimum

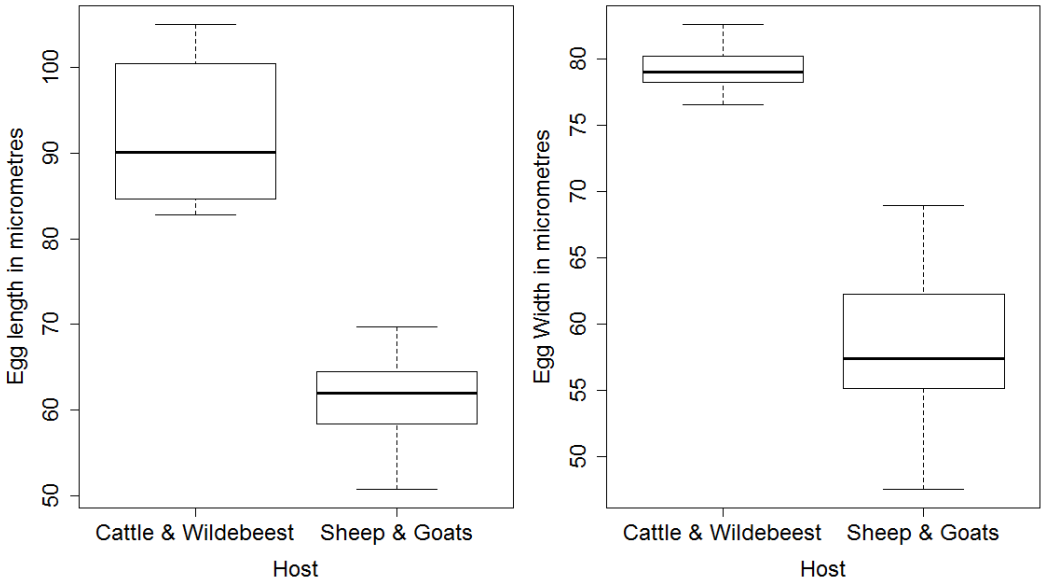


Figure 4.11: Box plots showing difference in mean length (left) and width (right) of *Moniezia benedini* eggs. The eggs were identified from cattle, wildebeest, sheep and goats.

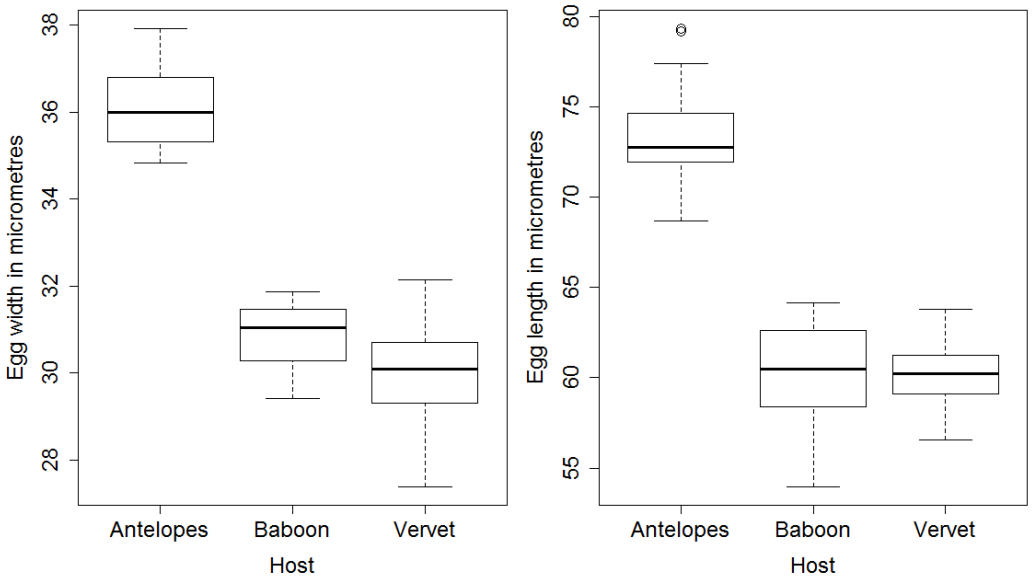


Figure 4.12: Box plots showing difference in mean length (left) and width (right) of *Trichuris* species eggs. The eggs were identified from Antelopes, Vervet monkeys and baboons.

4.3.5. Performance of faecal parasitological methods

Sedimentation and floatation were the two faecal parasitological methods used in this study for qualitative and quantitative identification of helminths and evaluation of helminths infection rates. The performance of the parasitological methods were evaluated on baboon samples only because baboon was the only host taxa that had faecal samples from the same individual in both dry and wet season. Results showed that the two parasitological methods differed in their performance, especially in the prevalence values (Table 4.2 and Table 4.5) and helminths species richness. Chi-square test showed that prevalence produced by floatation method were significantly higher ($\chi^2 = 157.472$, df = 1, $p<0.001$) compared to prevalence by sedimentation method across baboon social groups (Table 4.13; Figure 4.13). The concordance between floatation and sedimentation methods in terms of helminth prevalence across individual baboon samples was low as indicated by Cohens' kappa statistic (0.101).

Table 4.13: Observed frequencies of all infected and non-infected baboon samples collected in both wet and dry season

Parasitological method	Positive	Negative	Total
Floatation	217	65	282
Sedimentation	68	214	282

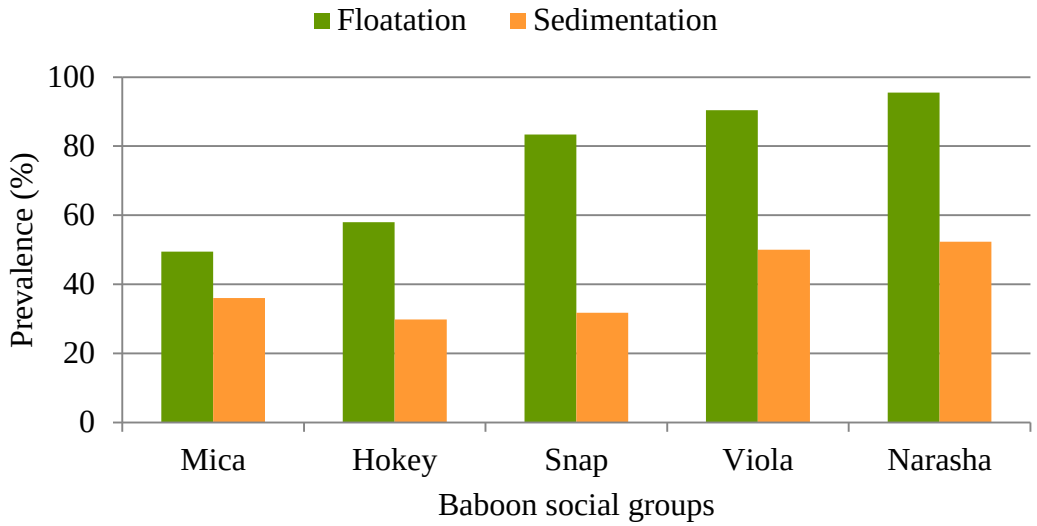


Figure 4.13: Prevalence of helminths identified by floatation and sedimentation methods in baboon population in Amboseli ecosystem, Kenya.

Moreover, prevalence of *Trichuris* sp. (37.2%) and strongylids (1%) by sedimentation method (Table 4.4) were lower compared to when determined by floatation method (Table 4.14).

Table 4.14: Prevalence of *Trichuris* sp. and Strongylids in baboons by floatation method

Wet season				**Dry season**		
Helminth species	***n***	**+ve**	**Prevalence (%)**	**+ve**	**Prevalence (%)**	**Mean prevalence (%)**
Trichuris sp	185	144	77.84	160	86.49	82.16
Strongylids	185	61	32.97	36	19.46	26.22

In terms of helminths species richness, results showed that except for strongylids which was detected by both coproscopic methods, there were five genera of helminths that were identified by sedimentation method (*Trichuris* sp., *Primasubulura* sp., *Spirurina* sp., *Enterobius* sp. and *Streptopharagus* sp.) while *Trichuris* sp. was the only genus identified by floatation method (Table 4.15). In contrast to prevalence, chi-square test showed that helminth species richness was significantly higher when fecal sedimentation method was used ($\chi^2 = 132.703$, df = 5, $p<0.001$) compared to fecal floatation method (Table 4.15).

Table 4.15: Observed frequencies of all samples in which different species of helminths were detected

	Number of positive baboon faecal samples by the two methods of analysis	
Parasite type	Sedimentation method	Floatation method
Trichuris sp	66	209
Strongylids	4	87
Spirurina sp	34	-
Streptopharagus sp	2	-
Primasubulura sp	3	-
Enterobius sp	1	-
Total	110	296

4.4 Discussion

4.4.1: Sedimentation parasitological method

4.4.1.1 Overall prevalence of helminths in the different host species

Grant's gazelle was the host species among the Amboseli sympatric host community that had the highest (97.9%) mean prevalence of helminths (Table 4.2) which was comparable to other Grant's gazelle populations in Kenya, such as those in Mpala Ranch, Laikipia County (Ezenwa, 2003; Ezenwa, 2004b). This could imply that Grant's gazelles are highly susceptible and could be a key reservoir of helminths in the host community.

The host which had the second highest prevalence in the sympatric host community was Thomson's gazelle that had 75% prevalence of helminths (Table 4.2). Thomsons' gazelle is also highly susceptible to helminths infections as prevalence in other populations in Kenya were recorded to be between 84% and 100% (Vanderwaal *et al*., 2014; Ezenwa, 2003).

Goat was the third most infected animal species among the sympatric host community with a prevalence of 73.9%. Interestingly, goat had the higher prevalence compared to the other domestic ungulates. Previous studies elsewhere have recorded even higher prevalences (Maichomo *et al*., 2004; Kanyari *et al*., 2009; Kumsa *et al*., 2011) compared to the current study, which suggests, free ranging goats are highly susceptible to helminths.

Prevalence of helminths in sheep (63.5%) was lower compared to prevalence of 80% that was previously recorded in flocks of sheep in Magadi, Kajiado County (Maichomo *et al*.,

2004). Prevalence of helminths in free-grazing sheep is generally high ranging between 81-100% in different regions (Maichomo *et al*., 2004; Kumsa *et al*., 2011).

Impala is one of the Antelope species that generally have high prevalence of helminths infections however, in the current study, prevalence of helminths (62.5%) was much lower compared to Grant's and Thomson's gazelles (Table 4.2). Moreover, the prevalence was also lower in comparison with other populations elsewhere (Nalubamba *et al*., 2012; Vanderwaal *et al*., 2014).

Prevalence of helminths in the Amboseli vervet monkey population (62.5%) was comparable to prevalence of helminths (68.9%) in a population inhabiting the semi-arid Radom National Park, South Sudan (Abuessailla, 2012). In contrast, prevalence of helminths was much lower (28%) in a population of vervet monkey at Segera Ranch, Laikipia, Kenya (Ekdahl, 2005). Prevalence of helminths seems to be lower in vervet monkey populations inhabiting arid and semi arid habitats compared to those in richer forested habitats. For instance, populations of vervet monkeys in the forested Kibale National Park, Uganda, were recorded as having prevalence of 92% (Gillespie *et al*., 2004) while those in the forest reserves of the Ethiopian Rift valley had prevalence of 92.7% (Legesse and Erko, 2004). It is possible that the cooler and wet environments in forested habitats facilitate longer survival and rapid development of pre-parasitic stages.

Prevalence of helminths in cattle was 56.2% (Table 4.2), which was higher compared to rates of prevalence that has been previously recorded in free ranging cattle in the region. For example cattle in Ol Pejeta Conservancy, Laikipia, Kenya and Ngorongoro District, Tanzania, had 20 - 21% prevalence of helminths (Swai *et al.,* 2006; Vanderwaal *et al*.,

2014). Elsewhere in Ethiopia, prevalence of helminths in free-ranging cattle was recorded at 53%, which dropped to 29% after deworming (Nnabuife *et al*., 2013). Nevertheless, other factors such as age structure ad seasonality are strong determinants of prevalence and could influence variations across locations.

Wildebeest was the animal species that had the least prevalence of helminths (38.5%) among the sympatric host community. This is the first record in Kenya of helminths prevalence in resident wildebeest.

This study reveals that in the Amboseli host community, Grant's gazelle was central to the epidemiology of helminths in the sympatric host community as its population was dominantly infected. However, its role in the epidemiology of helminths in the host community was not explicit. It was also interesting that the browser host community, which mainly comprised of the antelope species and goats, was more infected than the grazer host community, cattle, sheep and wildebeest. It is expected that since grazers are ground-level feeders, they have higher infection than browsers. The natural pool of water in the area, which was the only source of water for all the animals, could have been the possible source of transmission for the browsers and higher prevalence could fundamentally suggest greater susceptibility to most of the helminths genera compared to grazers.

In addition, this study reveals that although multiple hosts co-occur in a habitat and are exposed to similar species of environmentally transmitted helminths, the rates of helminth transmission or infection level significantly differ (χ^2 = 23.87, df = 8, p = 0.0001) across host species (Table 4.2). Variation in prevalence of helminths among

sympatric hosts is likely to be subject to multiple intrinsic and extrinsic factors such as dissimilarities in susceptibility to various helminth species (Gustafson *et al*., 2013), age and sex structure of host species, density, foraging behavior, immunity, mode of feeding and intensity of foraging (Vanderwaal *et al*., 2014).

Seasonality was also a strong factor that significantly influenced (χ^2 = 200.37, df = 1, p = 0.0001) the pattern of helminth infection across the sympatric host community. Except in baboon and Grant's gazelles, all hosts showed a pattern of higher helminth prevalence in the wet compared to dry season (Figure 4.2). Seasonality has dual effects on parasitism; host and parasite, whereby, seasonally driven – habitat resource availability or limitation may enhance or reduce host fitness to infection or survival and transmission of parasites (Coop and Kyriazakis, 1999). Generally, helminth infective stages proliferate under wet season characterized by the warm temperature and sufficient moisture unlike in dry season in which they suffer rapid dessication (Van Djik *et al*., 2009; Turner and Getz, 2010; Nalubamba *et al*., 2012). The higher prevalence of helminths in the dry season for both baboons and Grant's gazelles could be attributed to reduced fitness.

4.4.1.2 Prevalence of different helminths across hosts

The most prevalent helminth in Grant's gazelles was strongylids (95.8%), which is a group of nematode species. Since strongylids is an assemblage of different species of nematodes, the combined group effect coupled with their fecundity gives them dominance over other species of helminths. In comparison with populations elsewhere, strongylids were also found to be the most common and highly prevalent helminth in Thomson's gazelles (Ezenwa, 2003; Ezenwa, 2004b), which suggests that due to their group effect strongylids competitively dominate other helminths species. The other two

species identified, *Trichuris sp.* and *Strongyloides sp.*, had variable (49% and 2.1% prevalence), respectively (Table 4.3), which is consistent with studies elsewhere (Ezenwa, 2003).

Strongylids were also the most prevalent in the Amboseli population of Thomson's gazelle (60.45%), which was comparably higher than prevalence in a population at Ol Pejeta Conservancy, Laikipia, Kenya (Vanderwaal *et al*., 2014). Although prevalence of *Trichuris* sp. (35.4%) in Thomson's gazelle was low in the present study, it was comparably higher than prevalence of 5% found in a population at Ol Pejeta Conservancy (Vanderwaal *et al*., 2014).

In small domestic ruminants, strongylids were also the most prevalent helminths in goats (71.8%) followed by *Strongyloides* sp. (5.2%), while the rest (*Moniezia* sp. and *Spirurid* sp.) were less than 3% (Table 4.3). Similarly, the most prevalent helminth in sheep were strongylids (64.6%) while the rest (*Spirurid* sp., *Strongyloides* sp. and *Moniezia* sp.*)* having prevalence less than 5%. Previous study in the same area by Maichomo *et al*., (2014), showed comparable prevalence of Strongylids (72-75%) in sheep and goats to the present study but higher prevalence of *Strongyloides* sp. (43-45%). In comparison with herds of goats and sheep elsewhere, it suggests that strongylids are the most prevalent helminths in small domestic ruminants, which is consistent with observations by Kumsa *et al*., (2011). In the present study, other helminths such as *Strongyloides* sp. and *Moniezia* sp. occurred at low prevalence in both goat and sheep (Table 4.3), which is consistent with findings by Kumsa *et al*., (2011) in Ethiopian village herds.

In the present study, impala were mainly infected by strongylids with a prevalence of 70.8%. Previous studies have recorded high prevalence of strongylids (>80%) across populations of impala in five ecologically distinct habitats in Kenya (Ezenwa, 2004a; Vanderwaal *et al*., 2014), which indicates strongylids are highly prevalent in impala.

The most prevalent helminths in Amboseli vervet monkey was *Trichuris* sp. (53.2%) followed by *Primasubulura* sp. (29.1%). Other helminth species (*Enterobius, Spirurina,* and Strongylids) had low prevalence (< 20%). Prevalence of *Trichuris* sp. in vervet monkey is highly variable across populations (Muriuki *et al*., 1998; Mutani *et al*., 2003; Legesse and Erko, 2004; Gillespie *et al*., 2004; Kooriyama *et al*., 2012).

Prevalence of *Primasubulura* sp. and *Spirurina* sp. were higher in the vervet monkey population at Amboseli compared to prevalence recorded in populations in rich habitats such as the Mahale mountain National Park in Tanzania (Kooriyama *et al*., 2012). Both *Primasubulura sp.* and *Spirurina sp.* are transmitted through accidental or deliberate ingestion (insectivory) of their invertebrate hosts (Orthoptera – locusts and crickets and Coleopteran - beetles) by vervet monkeys and other non-human primates. Since, forest habitats are generally expected to host more abundance of insects, lower prevalence of these nematodes in vervet monkeys may imply that despite abundance of insects, insectivory may not be a significant dietary component in forest dwelling vervet monkeys as it is in an impoverished habitat like Amboseli ecosystem (Alberts *et al*., 2005). Insectivory is a common trait in non-human primates, but its frequency varies from low to high, both within and across taxa and across habitats (McGrew, 2014).

Relative to other host species, prevalence of strongylids was remarkably low (6.2%) in vervet monkeys (Table 4.3). Similarly, prevalence of strongylids in Amboseli vervet monkeys was comparably low to prevalence recorded in populations elsewhere in Kenya and Uganda (Muriuki *et al*., 1988; Gillespie *et al*., 2004). *Oesophagostomum* spp. is one of the strongylids that have been identified in vervet monkeys (Gillespie *et al*., 2004).

In cattle, strongylids were the most prevalent helminths (57.3%) while the rest (*Spirurid* sp. *Fasciola hepatica, F.gigantica* and *Paramphistomum* sp.) had low prevalence of between 3.2 - 5.2% (Table 4.3). Previously, studies in the same area by Maichomo *et al*., (2004) reported that Trichostrongylid and *Strongyloides* sp. were the most prevalent helminths. Elsewhere, strongylids have been reported to be the most prevalent helminths (Vanderwaal *et al*., 2014).

Little information is known about the unidentified species of spirurid nematode that was found in cattle except that it was identical to the type that was previously found in cattle at Ol Pejeta conservancy, Kenya (Vanderwaal *et al*., 2014) and that its prevalence in Amboseli herds (5.2%) was lower. In addition, the spirurid was also found in the Amboseli wildebeest, which suggests that it is a bovid spirurid.

The prevalence of strongylids in wildebeest was the highest (39.6%) compared to the other two helminths, *Fasciola hepatica* (2.1%) and *Spirurid* sp. (2.1%). However, the prevalence of strongylids was lower compared to prevalence (81%) recorded in other populations elsewhere such as those inhabiting the semi-arid Etosha National Park, Namibia (Turner and Getz, 2011).

Results from this study reveal that in the Amboseli host community, strongylids were the most dominant helminth among the ungulates, whereas *Trichuris sp.* was dominant among the non-human primates. This implies that helminth species may be numerous in a multi-host community but one or two species of helminths may dominate and shape the infection pattern.

4.4.1.3 Prevalence of helminths in baboons

Previous studies suggest that prevalence of helminths in baboons is strongly influenced by climatic conditions. Specifically it is suggested that prevalence of helminths is overally higher in baboon populations that inhabit cool climatic conditions (Muller-Graf *et al*., 1996; Legesse and Erko, 2004) compared to populations inhabiting semi arid, arid and desert regions (Appleton and Brain, 1995; Ghandour *et al*., 1995; Bezjian *et al*., 2008). These previous observations are consistent with the low prevalence of helminths that was recorded in the Amboseli baboon population 31.2% (Table 4.2). For the the past 23 years (1990-2012), Amboseli ecosystem has experienced very low annual mean rainfall (< 140mm) usually characterized by six months period of < 10mm of rainfall (Chapter 3: Figure 3.2). Climatic condition characterized by such low rainfall and high ambient temperature (Altmann *et al*., 2002) is anti-helmintic as it dessicates propagules and sanitizes the environment from infective stages (Appleton and Brain, 1995; van Dijk *et al*. 2009).

It was also observed that prevalence of helminths was significantly different across baboon social groups (Figure 4.3), which is not uncommon. A similar observation was recorded among five social groups of baboon inhabiting Gombe stream National Park, Tanzania (Muller-Graf *et al*., 1996). Despite the close proximity and even

geanealogically relatedness of three social groups in Gombe, inter-group difference in prevalence of helminths suggest group membership as a strong determinant of social clustering in prevalence. For example, the inter-group variation in age and sex structure is likely to influence rate of infection (Muller-Graf *et al*., (1996). Nevertheless, no single factor can explain drivers of inter-group variations in prevalence of helminths. Heterogeneity in micro-habitat conditions, patchy distribution of resources and infective stages, the dynamic, brief and evernescent overlap of essential resources with parasites are suggested as possible drivers for inter-group difference in prevalence of helminths (Muller-Graf *et al*., 1996).

Prevalence of helminths in the Amboseli baboon had a seasonal pattern that deviated from the pattern observed in all other sympatric host community (Figure 4.2) whereby, prevalence was significantly higher in the dry season compared to the wet season. Factors that could have caused such deviation were not obvious but likely to be seasonally driven host-specific trait that strongly influences transmission. During the dry season in Amboseli, the baboons shift their spatio-temporal foraging behavior as they spend more time in smaller habitats (Chapter 3:Table 3.1 - 3.4) digging up grass corms and roots compared to wet season when they prefer flowers, fruits and seeds (Alberts *et al*., 2005). Since helminths are faeco-orally transmitted whereas baboons use their fingers to dig, this behavior is likely to have enhanced transmission and prevalence of helminths during the dry season.

4.4.1.4 Prevalence of different helminths in baboons

The most prevalent helminth in the Amboseli baboon was *Trichuris sp.* at 37.2% followed by *Primasubulura sp.* at 24.3% while others (Strongylids, *Enterobius* sp.,

Streptopharagus sp.) were below 3% (Table 4.4). *Trichuris* sp. is one of the most common helminths in non-human primates that often occur at high prevalence across populations (Muller-Graf *et al*., 1996; Hahn *et al*., 2003; Bezjian *et al*., 2008; Kooriyama *et al*, 2010; Howells *et al*., 2011; Kooriyama *et al*., 2012; Ravasi *et al*., 2012). In the Amboseli baboon, *Trichuris* sp. has persisted over the years though in the present study its prevalence is relatively low compared to the prevalence of 72.9% that was recorded 18 years ago (Hahn *et al*., 2003). It is interesting to note how prevalence of *Trichuris* sp. changes over time in baboon populations. Ebbert *et al*., (2013) found that prevalence of *Trichuris* sp. (28.2%) determined in 1979 in Mt. Assirik population, Ethiopia, had increased to 85.4% in the year 2000.

The subulurid nematode, *Primasubulura* sp. was only present in baboons and vervet monkey (Table 4.3), which suggests it is non-human primate subulurid. Previously, the species found in red-tailed monkeys was identified as *P. distans* (Kooriyama *et al*., 2010) and was also found in sympatric baboons and vervet monkeys of Mahale National Park, Tanzania (Kooriyama *et al*., 2012). Prevalence of *Primasubulura sp.* (24.3%) in Amboseli population (Table 4.4) was higher compared to prevalence in baboon population (5%) at Mahale National Park, Tanzania (Kooriyama *et al*., 2012). This is the first recod of *Primasubulura sp.* in Kenya.

Presence of *Streptopharagus sp.* in Amboseli baboon suggests that the helminth has persisted for years in the host population. This is because the helminths species has persisted for nearly 29 years in the Amboseli baboon population, in which its prevalence by then was < 2% (Meade, 1983). Later, the prevalence of *Streptopharagus sp.* in the Amboseli baboon population was recorded to be 16.5% (Hahn *et al*., 2003), which is

much higher compared to earlier records and present prevalence of 1% (Table 4.4). Across baboon populations inhabiting varied habitas, prevalence of *Streptopharagus sp.* is highly dissimilar and can be as high as 80% (Appleton and Bain, 1995; Muller-Graf *et al*., 1996).

The insect-borne *Spirurina sp.* was less prevalent (2.1%) in baboons compared to the vervet monkeys (10.4%), which was the only other host that harboured the nematode (Table 4.4). Elsewhere, baboon populations in Mahale National Park, Tanzania (Kooriyama *et al*., 2012) had higher prevalence of *Spirurina sp.* (51%) compared to prevalence in the present study.

Interestingly, the three helminths, *Primasubulura sp., Streptopharagus sp. and Spirurina sp.* are insect-borne nematodes that are transmitted via insectivory. This means that the epidemiology of these nematodes may be associated with frequency of insectivory, which concurs with the suggestion by McGrew, (2014) that frequency of insectivory in non-human primates is heterogenous across troops, host taxa and geographical locations.

Strongylid nematodes are usually the most prevalent helminths in any host community (Ezenwa, 2003; Ocaido *et al*., 2004) and across host taxa (Table 4.3) but the baboon population in the present study had prevalence of 1% which was low compared 23.5% previously recorded by Hahn *et al*., (2003) in Amboseli baboons. Prevalence of strongylids in baboon populations is highly variable (Muriuki *et al*., 1998; Hahn *et al*., 2003; Muller-Graf *et al*., 2003; Legesse and Erko, 2004; Kooriyama *et al*., 2012) which means it cannot be attributed to a single factor.

The eggs of *Enterobius sp.* are usually rare in faeces because gravid worms lay eggs that attach on the peri-anal region to facilitate rapid re-infection. This could explain the very low prevalence of *Enterobius sp.* (0.5%) in the present study baboon population and probably its absence in previous assessment by Hahn *et al*., (2003) in Amboseli and in Gombe stream population (Muller-Graf *et al*., 1996). Overall, prevalence of *Enterobius sp.* in wild baboon populations is generally low (< 15%) across habitats (Ocaido *et al*., 2003; Hahn *et al*., 2003).

4.4.1.5 Prevalence of helminths in ungulates and non-human primate host groups in Amboseli ecosystem.

Vervet monkeys and baboons were the non-human primates among the sympatric host community in Amboseli. In many African habitats, these species of non-human primates tend to co-occur. In the present study, both vervet monkeys and baboons were infected with gastrointestinal helminths but infection prevalence in the vervet monkeys was significantly higher compared to infection in baboons. Difference in prevalence of helminths between overlapping closely related hosts or social groups is not new. Chapman *et al*., (2005) observed variation in prevalence of *Trichuris sp.* between overlapping populations of two species of Colobus monkey. Factors driving such difference in prevalence of helminths have been attributed to variations in host intrinsic factors (immunity, sex, age, co-infections), population density, stress, habitat modification, group size, ranging size and divergent behavioral ecology (Marsh, 1981; Chapman *et al*., 2005; Mbora and McPeek, 2009).

Wild and domestic animals seemed to share most of the helminths species and so in areas of overlap there is likely to be sharing, which explains the observed lack of difference in prevalence between the domestic and wild conspecifics.

Ungulate host group had significantly higher prevalence of helminths compared to the prevalence in non-human primates (Figure 4.4), which may be associated with dissimilarity in ancestry. This is because hosts with common ancestry (closely-related) tend to have similar foraging patterns, nutritional demand, behavioural, ecological, physiological and spatial space use, traits that may facilitate similarity in parasite exposure, susceptibility and mechanisms of immune defense (Page, 2003; Woolhouse *et al*., 2005; Wolfe *et al*., 2005; Wolfe *et al*., 2007; Davies and Pedersen, 2008).

The seasonal pattern of prevalence was variable across the three host groups. The pattern of infection in both domestic and wild ungulates was characterized by higher prevalence of helminths in the wet than dry season (Figure 4.4). Although, prevalence of vervet monkey was higher in the wet than in dry, when combined with baboon, the prevalence of helminths in non-human primates was higher in the dry than wet season. Such structured seasonal patterns in helminth infection may suggest that climatic factors that determine transmission are likely to supersede other determinant factors such as phylogenetic dissimilarities.

4.4.2: Floatation parasitological method

4.4.2.1: Helminth prevalence in all sympatric host species

Most of the studies that have recorded prevalence of helminths in different animal hosts based their findings on sedimentation method or combined results that were obtained by both sedimentation and floatation parasitological methods. As such, the results on prevalence of helminths based on floatation method alone are exclusive to this study and not comparable to values obtained in previous studies.

Overall, in the present study, prevalence of helminths across all the sympatric hosts was significantly different. The gazelles were the most infected host taxa while the monkeys were the least (Table 4.5 and 4.6), which suggests that transmission of helminths was heterogenous across taxa of hosts even those in sympatry, meaning that intrinsic factors that influence transmission are unique across hosts and supersede the effects of spatial host convergence.

The effect of seasonality on prevalence of helminths across hosts was strongly influenced by either inclusion or exclusion of baboon as a host. Specifically, when prevalence of helminths in baboon was included with prevalence of all other hosts, the effect of season was nullified and prevalence of helminths across hosts were not statistically significant between dry and wet seasons, which could be basically the effect of the large sample size of baboon (n = 370) as a host viz a viz sample size (n = 96) of each of the rest of the sympatric hosts (Table 3.5).

4.4.2.2: Prevalence of helminths across baboon social groups

The baboon social groups also displayed statistically significant variation in prevalence of helminths (Table 4.9), which could be attributed to group-specific disposition to

transmission of helminths. Interestingly, prevalence did not significantly differ between wet and dry seasons across social groups, which may suggest that climatic conditions did not influence transmission. However, this interpretation is to be taken with caution as it is based on floatation method, which is rarely used as a stand alone faecal assessment method.

4.4.3 Abundance of helminths

4.4.3.1 Abundance of helminths in all sympatric hosts in Amboseli ecosystem

In the Amboseli host community, *Trichuris* sp. and Strongylids were the two most abundant helminths. Since abundance was not based on eggs per gram (epg), which is a common measure of abundance in many studies, the present results could not be compared with other populations elsewhere. However, in the present study, an interesting pattern was observed whereby the abundance of Strongylids was highest in goat, sheep and Grant's gazelle whereas abundance of *Trichuris* spp. was highest in vervet monkey, baboon and Thomson's gazelle (Figure 4.5). The abundance of strongylids and *Trichuris sp.* were similar only in Grant's gazelle (Figure 4.5), whereas in other hosts, abundance of the two helminths species was dissimilar. Competition between co-occuring parasites in a host is inevitable because resources within a host are limited and such competition may limit proliferation of the other parasite species (Lello *et al*., 2004).

Although, abundance is not a perfect indicator of the number of worms in a host, it serves as an indicator of the level of infection. In the study host community, the mean abundance (wet and dry season) in decreasing order shows that vervet monkey had the highest level of infection (119.7) followed by goat (115.7), Grant's gazelle (91.2), Thomson's gazelle (78.5), baboon (56.4), sheep (56.2), cattle (16.8), wildebeest (13.1)

and lowest being impala (10.6). It is interesting that impala had the least abundance in the present study and yet in Kruger National Park, South Africa, 17 species of nematodes totaling 1.3 million worms were recovered from impala population (Negovetich *et al*., 2006).

4.4.3.2 Abundance of helminths in baboons

Strongylids and *Trichuris sp* were the two helminth species whose abundance was determined in baboons. However, *Trichuris sp.* was the most abundant (49.6) of the two whereby abundance of strongylids was very low (0.61). Such difference in abundance between helminths species may be due to difference in their mode of transmission. Strongylids are transmitted through infective larval stages whereas the embryonated egg of *Trichuris* sp. is infective. The combination of harsh climatic conditions and the dry season shift in foraging approach by baboon especially in a dry and hot environment like that of Amboseli, may result in low transmission of strongylid nematodes compared to *Trichuris* sp.

Abundance of helminths varied across social groups (Figure 4.6). Host age is a strong determinant in the infection pattern of *Trichuris sp*. since highest intensity of infection in humans occurs at ages (5- to 9-yr-old) while in baboons, the highest infections occur at age extremes, <1- or >15-yr-old (Anderson *et al*., 1993; Anderson *et al*., 2012). In the present study, age-structure across social groups was unknown but results of the abundance could suggests that Narasha and Viola, which had the highest abundance (> 115), have majority of very young or older individuals. It was interesting to note that there was no structured pattern in abundance across baboon social groups (Figure 4.6). This may mean that although seasonal climatic changes determine abundance of

helminths, variations in intrinsic factors of social groups is likely to be a central factor that drives the differences in abundance of helminths across social groups.

4.4.4 Helminth species richness in all hosts

Parasite species richness (PSR), which is the pool of all parasites infecting a given host community at a given point in time, is referred to as the component community (Poulin, 2004). In the present study, the component community consisted of 16 species of helminths (Table 4.11), which was higher compared to (n = 8) host communities at both Mpala Ranch and Ol Pejeta conservancy, Kenya (Ezenwa, 2003; Vanderwaal *et al*., 2014). The component community based on mature worms recovered by necropsy from sympatric wild bovid community in Mpala Ranch, Kenya was however higher (n = 15) compared to the present study, which suggests that PSR is likely to be under estimated by parasitological methods of helminths egg assessment (Ezenwa, 2003; Ocaido *et al*., 2004). The sensitivity of parasitological methods could be because some helminths have low egg fecundity and output in faeces as well as slow larval development.

The average component community for the Amboseli host community (N = 9) was 5.1 ±1.9 (Table 4.11), which was higher compared to 3.6 ±1.5 the bovid host community (N = 11) at Mpala Ranch, Kenya (Ezenwa, 2003) probably due to a more diverse host community in Amboseli compared to that of Mpala which were mainly bovid antelopes (browsers). This means that component community is most likely a function of host phylogeny whereby sympatrism that involves unrelated hosts is likely to beget more helminth richness. Moreover, in the present study, the inclusion of livestock further enriched the component community of the Amboseli sympatric hosts because of

livestock's vagility. Usually, the component community is expected to be variable and dynamic as some parasite species become locally extinct, while it increases as nearby host populations become colonized (Poulin, 2004).

At a much smaller scale, PSR in individual host taxa, refered to as infracommunity, is also expected to be variable, being subject to multiple intrinsic and extrinsic factors. Hosts range of diet, vagility and relatedness to other sympatric host species and how long ago it arrived in an area are some determinants of infracommunity (Kennedy *et al*., 1986; Kennedy and Bush, 1994).

In the present study, infracommunity ranged from 2 - 8 (Table 4.5), meaning that all host taxa had at least one type of helminth species. This was comparable to infracommunity range of 2 - 6 in the bovid host community at Mpala Ranch, Kenya (Ezenwa, 2003). Since average PSR for individual host taxa was 5.1, hosts with PSR higher than the average such as baboon, cattle, sheep and goat (Table 4.11) were considered to have high PSR. The average PSR for domestic ungulates (6.3 ± 0.58) was higher compared to that of wild ungulates (3.5 ± 1.29) though non-human primates (6.5 ± 2.12) had the highest (Table 4.11). This suggests that livestock in Amboseli ecosystem harbor more species of helminths compared to wild ungulates meaning that livestock are exposed to more diverse helminths and are likely to pose risks of introducing other helminths species to the wild conspecifics. The role of range size on PSR is rather controversial and inconclusive, but in the present study, the extensive ranging by the pastoralist livestock could be theoretically linked to the higher PSR.

Populations of impala, Grant's gazelle and Thomson's gazelles in the present study had PSR of two, three and four respectively, which are comparably lower to PSR (six, six and five, respectively) of their conspecifics at Mpala Ranch, Kenya (Ezenwa, 2003). In Ol Pejeta Conservancy, Kenya, impala population had PSR of three (Vanderwaal *et al*., 2014) which susggests that PSR in impala is relatively low in Kenyan populations. Thomson's gazelle in Ol Pejeta Conservancy and Mpala ranch, both in Lakipia County, Kenya, had PSR of five (Ezenwa, 2003; Vanderwaal *et al*., 2014). Impala population at Lake Mburo National Park, Uganda had PSR of 15 mature helminths that was recovered by necropsy (Ocaido *et al*., 2004).

Cattle herds in Ol Pejeta Conservancy had PSR of two (Vanderwaal *et al*., 2014), which is much lower compared to the PSR of seven in the present study (Table 4.11). In contrast, PSR in livestock determined by mature stages of worms recovered by necropsy are always higher. For instance, Ankole cattle and goats that graze in wildlife areas of Lake Mburo National Park, Uganda had PSR of 13 and 10, respectively (Ocaido *et al*., 2004).

The PSR of a baboon population can be as low as three, which was reported in the Namib Desert (Appleton and Brain, 1995) to as high as 13, which was recorded in a population at Mt. Assirik, in the Niokolo-Koba National Park, Republic of Senegal (Ebbert *et al*., 2013). Several baboon populations have PSR of 5-8 (Munene *et al*., 1998; Ocaido *et al*., 2003; Legesse and Erko, 2004; Ravasi *et al*., 2012a; Ebbert *et al*., 2013). In Natal, South Africa, baboon populations in Drakensberg Mountain had PSR of seven while populations at the coastal region had 11. Previous PSR in the Amboseli baboon population was five (Hahn *et al*., 2003). These results are within comparable range of

PSR in the present study and suggest that combined climatic and habitat characteristics that promote survival of helminths could be important for PSR in baboon. The population of vervet monkey in Amboseli had higher PSR (5) compared to that in the Ethiopian Rift valley where only two helminth species were detected (Legesse and Erko, 2004).

Empirical theory suggests that infracommunity of parasites is on average predicted by host body size whereby larger bodied animals harbour higher helminths species richness compared to smaller-bodied animals across related hosts (Poulin, 1995; Morand, 2000). This is argued that host body size is a corresponding measure of available habitat size for parasites, which means that larger bodied hosts provide larger areas or volumes for parasite colonization in terms of all their organs, they offer more niches, live longer and are exposed to more parasite colonization rates (Poulin, 1995; Poulin, 1998; Morand, 2000). For instance in the present study, baboon and vervet monkey are phylogenetically related and baboon as the larger-bodied monkey had higher PSR (8) compared to vervet monkey (5).

It is also suggested that it is inappropriate to compare helminth species richness among sympatric host communities from different geographical regions without also taking into account their phylogenetic relatedness (Poulin, 2001). Host species from the same phylogenetic family are likely to have inherited certain helminth lineages from their common ancestor, which means that their helminth communities will tend to show similarities whether or not they now inhabit the same geographical area (Poulin, 1998; Poulin, 2001).

Although some of these arguments attempt to explain determinants of PSR, no single factor is adequate and universal thus new suggestions to explain drivers of infracommunity are being explored; for instance, genome anomalies are thought to have a role (Guegan and Morand, 1996; Poulin *et al*., 2000).

Helminths are ubiquitous and comprise of a great diversity of gastric parasites with an enormously wide host range. In the present study, 16 types of helminths that belong to three major groups; nematodes cestodes and trematodes were identified. Most of the helminths were nematodes followed by trematode and a single cestode (*Moniezia* Spp).

The pinworms, *Enterobius sp.* eggs (Figure 4.7A) are rarely seen in faecal matter because they are usually deposited on the peri-anal skin where they develop into infective larvae that eventually re-infect the host (Kucik *et al*., 2004). In the present study, pin worms were only found in vervet monkey and baboon and the mean egg size (n = 10) was larger (58.9 ± 1.1 by 28.8 ± 1.1) compared to 38 ± 2.8 by 24 ± 3.0 of the eggs of *Enterobius sp.* (n = 115) that were isolated in Senegal baboon (Ebbert *et al*., 2013). The size of the eggs in the present study were within comparable range of *E. vermicularis* (50-60 by 20-30µm) from humans (Jyothi *et al*., 2012), which suggests that the species in Amboseli non-human primates could be *E.vermicularis*. Infection by *E. vermicularis* is of public health interest because it is a zoonotic nematode that cause mild to fatal infection in old world non-human primates (Yaguchi *et al*., 2014), gastroenteritis in children (Jardine *et al*., 2006), and sometimes lead to vulvovaginities, endometriosis and perineal pruritis in women (Jyothi *et al*., 2012; Powell *et al*., 2013).

Strongyloides (Figure 4.7B) co-occurred in four different host species spanning the ungulate and non-human primate taxa (Table 4.11). The species common in non-human primates are *Strongyloides fuelleborni* and *S. stercoralis*. Hundreds of millions of people get infected by *S. stercoralis* (Anderson *et al*., 2012) though other strains of *S. fuelleborni* are also infectious to humans (Smith *et al*., 1991). Since the nematode co-occurred in both ungulate and NHP, it is possible that other *Strongyloides* species, such as *S.westeri* and *S. papillosus,* were present within the host community.

Strongylids, which represents a group of parasitic nematodes in the Order Strongylida and whose eggs are morphologically indistinguishable (4.7C), were the most co-shared across host taxa. The strongylids co-occurred in all the nine hosts, although (Table 4.11), there was no evidence whether the eggs in all the hosts were from one or more than one species of strongylid nematodes. Nevertheless, the result suggests that strongylids were the most co-shared nematodes in the present host community (Table 4.11). This is consistent with studies elsewhere in which strongylids were common in a host community that included cattle, goats, impala, zebra, waterbuck, topi, buffalo, reedbuck and oribi Ocaido *et al*., (2004). Similarly, Vanderwaal *et al*., (2014) also found that strongylids were the most co-shared helminths among eight sympatric hosts in Ol Pejeta conservancy, Kenya.

The second most co-shared genus of nematode was *Trichuris* with one egg morphotype, *Trichuris-otu-d* (Figure 4.8A) co-occurring in the two non-human primates, baboon and vervet monkey (Table 4.11) while the other morphotype *Trichuris-otu-c* (Figure 4.8D), co-occurred in five ungulate hosts of both wild- and domestic- groups (Table 4.11). These two *Trichuris* morphotypes were statistically different in size (Table 4.12; Figure

4.12) which could signal genetic variation. For instance, in South Africa, two *Trichuris sp.* egg-morphotypes (55 by 25.4 µm and 64.5 by 30.9µm) were isolated from two sympatric baboon troops (Ravasi *et al*., 2012b). These *Trichuris sp.* eggs were differentiated by size but after molecular analysis of mature worms isolated from the two troops, it was confirmed that the eggs represented two genetically distinct lineages of *Trichuris* species that were potentially zoonotic (Ravasi *et al*., 2012b).

The egg size of *Trichuris sp. otu-d* that was co-shared by the non-human primates in the present study was slightly larger (60.1±1.7µm by 29.9±1.2µm) compared to the mean egg size (50-55µm by 22-24µm) of *T. trichuris* (Stephenson *et al*., 2000). This means that the *Trichuris sp.* in the Amboseli baboon and vervet monkey was possibly not *T. trichuira* which is major public health burden as it infects > 500 million people worldwide (Liu *et al*., 2012). The egg size of *Trichuris* sp. in the baboons and vervet monkeys from Amboseli were however comparable to eggs of *Trichuris* sp. from South African baboon (Ravasi *et al*., 2012b), which suggests that the species is mainly harboured by non-human primates. The eggs of *Trichuris* sp. from the Amboseli ungulates were much larger (Table 4.12) compared to those in non-human primates, which suggests that the two egg morphotypes of *Trichuris* were distinct species that were co-occuring within sympatric host community but restricted within particular host taxa.

In the present study, there were two unidentified species of spirurids of which Spirurid-morphotype A occurred among five ungulates (Table 4.11) whereas Spirurid-morphotype B occurred in baboon only (Table 4.3). Spirurid- morphotype A (Figure 4.9C) has been reported in African buffalo in Kenya (Vanderwaal *et al*., 2014), suggesting that it is a bovid spirurid. Baboons tend to harbor diverse species of spirurids, though spirurid-

morphotype B was of unknown species (Figure 4.9D). The Amboseli baboon also harboured a rare spirurid in the genus *Spirurina* (Figure 4.9A), which previously was identified in several non-human primates inhabiting Mahale National Park, Tanzania (Kooriyama *et al*., 2012). Species of spirurids that is frequently reported in baboon and other non-human primates are *Physaloptera* and/or *Abbreviata* (Jessee *et al*., 1970; Poinar and Quentin 1972; Dewit *et al*., 1991; Muller-Graf *et al*., 1996; Bezjian *et al*., 2008; Howells *et al*., 2011; Kooriyama *et al*., 2012). However, it is highly probable to confuse eggs of *Spirurina* (Spiruroidea) *Physaloptera* (Spiruridae) and *Streptopharagus* (Spirocercidae) because the three egg types are elliptically identical with thick shells and have developing embryo. The distinctive feature across the three egg types is their size differences. The eggs of *Spirurina* (Figure 4.9A) are larger (Table 4.12) compared to *Physaloptera* while *Streptopharagus* (Figure 4.9B) is relatively the smallest (Jessee *et al*., 1970; Poinar and Quentin, 1972; Baker, 2007; Bezjian *et al*., 2008: Kooriyama *et al*., 2012). In the present study, unknown species of *Spirurina* and a *Streptopharagus sp.* were isolated from the Amboseli baboon while *Physaloptera or Abbreviata* were absent. In contrast *Physaloptera sp.* was prevalent some 18 years ago in the relatives of the current baboon population (Hahn *et al*., 2003), hence the absence of this nematode could imply 1) parasite community change over time or 2) the eggs previously identified as *Physaloptera* sp. were confused with those of other nematode eggs with similar morphological traits, particularly *Spirurina* sp. A similar observation was observed in baboon populations inhabiting Mt. Assirik in which *Physaloptera sp.* was present in the 1976-79 survey but were absent in the survey of the year 2000 (McGrew *et al*., 1989; Ebbert *et al*., 2013). *Abbreviata* sp. is also another nematode commonly identified in

baboons but its eggs can easily be confused with the eggs of rodent spirurid, *Protospirurira muricola*, which is much larger than *Spirurina* and *Abbreviata* (Kuntz and Myers, 1966; Petrlzelkova *et al*., 2010). This means that some of the previous records of *Physaloptera* and *Abbreviata* that did not include egg size ought to be reviewed or inferred with caution (Kooriyama *et al*., 2012). The detection of *Primasubulura* (Subuluroidea) and *Spirurina* (Spiruroidea) in the Amboseli population is the first record for these nematodes in Kenya even though they have been reported in several species of non-human primates elsewhere (Yamashita, 1963; Kooriyama *et al*., 2010; Kooriyama *et al*., 2012).

The cestode eggs of *Moniezia expansa* (Figure 4.8B) and *M. benedini* (Figure 4.8C) are known to co-occur in cattle, sheep and goats however *M. benedini* tends to be dominant in cattle (Nguyen *et al*., 2012). In the present study, *M. benedini* co-occurred in cattle, wildebeests, sheep and goats but those in cattle and wildebeests were significantly larger compared to those in goats and sheep (Figure 4.11). This could imply that *M. benedini* may be demonstrating genetic-structuring according to host taxa as has been observed in *Oesophagostomum bifurcum* (van Lieshout *et al*., 2005). Ocaido *et al*., (2004) identified *Moniezia* sp. in sympatric cattle, goats, waterbuck, buffalo and impala and perhaps assumed that the species is single, yet with egg measurements as in the present study, it is possible to tease infraspecies genetic variants. In contrast, *M. expansa* co-occurred between sheep and goats only, which agree with the findings of Nguyen *et al*., (2012) that also recorded co-occurrence of the nematode in the two hosts in Vietnam.

Among the trematodes, *Fasciola hepatica* (Figure 4.9F) was shared between cattle and wildebeest while *F.gigantica* and *Paramphistomum* sp. (Figure 4.9E) were present only

in cattle. Both *F. hepatica* and *F. gigantica* are important water-borne zoonotic parasites of both veterinary and medical importance (Tolan, 2011). The *Paramphistomum* spp. are multi-host digenean trematodes that infect a wide range of domestic and wild hosts, such as cattle, goats, impala, buffalo, waterbuck, zebra, warthog, and eland (Ocaido *et al*., 2004). It is interesting that in the present study, *F.gigantica* and *Paramphistomum* sp. were only found in cattle, irrespective of the presence of other susceptible sympatric hosts. This may imply either complete absence of *Paramphistomum* sp. and *F.gigantica* in other hosts or very low infection not detectable by the parasitological techniques used in this study.

The presence of *Macracanthorhynchus hirudinaceus* (Figure 4.10A) and *Protofasciola robusta* (Figure 4.10B) in baboons may be considered spurious infections. This is because baboons are not natural hosts for these helminths. The nematode in the genus *Macracanthorhynchus* are Acanthocephala which naturally infect suids including wild boar and warthogs (Mowlavi *et al*., 2006). The incidence of human cases with *M. hirudinaceus*, is however high in Asia (Mowlavi *et al*., 2006) which could also imply that host range for the helminth is likely broader than earlier thought. The eggs of *Macracanthorhynchus* are quite similar to the eggs of *Prosthernochis sp* (Acanthocephala) except that the former is larger (90-110µm long by 50-56µm wide) than the latter (65-81µm long by 42-53µm wide) (Baker, 2007). Even though both Acanthocephala species are transmitted through ingestion of infected beetles and cockroaches, *Prosthernochis* sp. is found only in new world non-human primates (Parr *et al*., 2013). In the present case, it is likely that baboons were accidentally infected through insectivory or through hand to mouth contamination with suid (warthog) faecal matter.

Presence of *P. robusta* in baboons may be associated with copro-feeding of elephant dung (Alberts *et al*., 2005). This is because *P. robusta* is a stomach trematode that specifically infects savannah elephants in Kenya (Obanda *et al*., 2011).

4.4.5. Performance of faecal parasitological methods

Qualitative assessments of helminth infection (prevalence and species richness) are often carried out by sedimentation method whereas quantitative analysis is often by floatation method. In the present study, both techniques were used for assessment of helminth infections in all the sympatric hosts in Amboseli ecosystem. Since baboon was the only population that individuals were known, thus only the samples from baboon individuals collected in both wet and dry season was used to evaluate performance of the parasitological techniques.

Therefore, based on baboon samples, results revealed that floatation method was more efficient for prevalence assessment as it yielded statistically higher prevalence compared to sedimentation methods (Figure 4.13; Table 4.13). In contrast, sedimentation method was efficient for evaluating helminth species richness (Table 4.15) because it recovered statistically higher helminth richness (n = 6) compared to richness (n = 2) by floatation method (Table 4.15). The results from this evaluation suggest the importance of using both techniques in faecal assessment studies.

CHAPTER FIVE: INFLUENCE OF HABITAT OVERLAPS AMONG BABOON GROUPS AND BETWEEN BABOONS AND ALTERNATIVE HOST SPECIES ON HELMINTH INFECTION PATTERNS

5.1 Introduction

Ecological factors that increase contact rates between susceptible hosts and infective stages of parasites are of epidemiological importance as they modulate transmission and patterns of infection (Arneberg, 2002). In free-ranging systems, it is a challenge to observe or directly measure contacts between individuals and groups, hence contact rates tend to be inferred from metrices such as degree to which two habitats of individuals, groups, or species overlap (Roemer *et al*., 2001; Schauber *et al*., 2007; Jimenez, 2007). Habitat overlap is considered an excellent ecological measure of both intra-species and inter-species contact rates while social units of a population such as host density and group size serve as proxy for intra-species contact rates (Cote and Poulin, 1995; Arneberg *et al*., 1998; Ezenwa, 2003; Arneberg, 2001).

The association between infection parameters and level of habitat overlap has been tested previously, with variable outcomes. For instance, nematode prevalence has been positively associated with increased habitat overlap among sympatric wild bovids in Kenya (Ezenwa, 2003). However, such association has not been tested on a host community that includes both livestock and livestock and hosts from diverse ancestry. Morever, it is not known how degree of habitat overlap within social groups affects helminths infection parameters. In addition, it is not clear whether animals that inhabit patches with more conspecifics or heterospecifics harbour more or less helminths infection rates. Therefore, this study aimed at specifically testing the effect of the degree

of habitat overlap between baboon groups and helminth prevalence, abundance and species richness in the Amboseli baboon population. Similarly, the effect of degree of habitat overlap between baboon and alternative host species that co-occur in baboon ranges was tested.

5.2 Material and Methods

This section will specifically test the associations between degrees of habitat overlap between baboon social groups and between baboons and alternative hosts, which was determined in chapter 3, section 3.3 and helminths infection parameters, which were determined in chapter 4, section 4.3.

5.2.1 Effect of the degree of habitat overlap on helminth infection in baboon groups.

Linear regression was used to test the association between degree of habitat overlap (proportion of shared area) and helminth prevalence, abundance and species richness across baboon social groups. The effect of social structures such as home range, group size and group density on helminth prevalence, abundance and species richness (Table 5.1) were also tested. Specifically, Spearman rank test was used to test whether there was a relationship between mean helminths prevalence, abundance and species richness (dry and wet season) of a baboon social group and 1) the total group size 2) baboon density at different levels of core habitat (50% MCP and 100% MCP). All statistical significance was considered at $p = 0.05$.

Table 5.1: Variations in baboon group size, density, mean helminth abundance and prevalence

Baboon group	Population measures			Helminth infection		
	Size (*N*)	Density (50% MCP)	Density (100% MCP)	Mean abundance	Mean prevalence (%)	Mean species richness
Narasha	43	2.88	7.83	126.9	52.3	5
Snap	28	2.38	1.28	12.8	50	4
Mica	34	1.2	2.18	14.5	36	2
Viola	64	13.74	2.85	119.1	31.8	3
Hokey	72	0.9	7.26	28.3	29.8	3
Weaver	117	3.74	3.19	36.9	19.2	

5.2.2: Effect of the degree of habitat overlap between baboon and alternative hosts on helminths infection in baboons

5.2.2.1 Dung pile counts as index of overlap

Mean prevalence, abundance and species richness of helminths for each baboon social group and mean dung counts are listed in Table 5.2. The association of dung pile counts on mean prevalence, abundance and species richness of helminths across baboon groups was tested using linear regression for each home range at 50% and 100% MCP.

Table 5.2: Mean prevalence and abundance of helminth infection in baboons and degree of overlaps at 50% and 100% MCP of baboon group's habitats

Baboon social group	Helminth infection			Index of dgree of habitat overlap	
	Mean abundance	Mean prevalence (%)	Mean species richness	Mean dung count (50% MCP	Mean dung count (100% MCP)
Narasha	126.9	52.3	5	8.5	65.6
Snap	12.8	50	4	21.6	137
Mica	14.5	36	2	12.6	78.4
Viola	119.1	31.8	3	18.2	78.3
Hokey	28.3	29.8	3	40.9	115.9
Weaver	36.9	19.2	2	33.5	90.2

5.2.2.2 Frequency of animal sightings as index of overlap

To estimate the influence of alternative hosts species on patterns of helminth infections in baboons, frequency of sightings of alternative hosts, here used as an index of host presence, was determined in baboon group home ranges at 50% MCP and at 100% MCP. First, Shannon-Wiener diversity index of the alternative hosts in the home range of a baboon groups was calculated from the number of sightings of each hosts species in the wet and dry season. The Shannon-Wiener index is one of the mathematical measures of biodiversity in a community which accounts for both abundance and evenness of the species present, whereby evenness is a measure of how similar the abundance of different species are (Spellerber and Fedor, 2003). Its advantage is that it provides more

information about community composition than simply species richness, which is the number of species present (Spellerber and Fedor, 2003).

Linear regression was performed between independent variables (helminth prevalence, abundance and species richness in baboons), and predictor variables [alternative host diversity (number of different host species sighted in a baboon home range), Shannon-Wiener diversity index of host species and mean frequency of all alternative host species sighted].

5.3. Results

5.3.1 Effect of the degree of habitat overlap on helminth infections in baboon groups.

The results indicated lack of statistical association (p = 0.05) between all helminth infection variables and measures of degree of habitat overlap at 50% MCP (Table 5.3). The social structures investigated for their influence on helminths infection were home range size, group size and density (Table 5.4). The analyses revealed that home range size at 100% MCP had a statistically significant association with prevalence (r^2 =0.758, $F_{(1,4)}$ =12.53, p = 0.024) and helminth species richness (r^2 =0.954, $F_{(1,4)}$ =83.81, p = 0.001) in the dry season more than expected by chance (Table 5.4).

Table 5.3: Relationship between degree of habitat overlap across baboon groups and helminth infection.

Variables	r^2	F statistics	P-value
Proportion of shared area and helminths infection in the wet season			
Prevalence versus proportion of shared area at 50% MCP	0.0365	2.989	0.204
Abundance versus proportion of shared area at 50% MCP	0.065	0.280	0.625
Helminth richness versus proportion of shared area at 50% MCP	0.588	5.669	0.075
Proportion of shared area and helminths infection in the dry season			
Prevalence versus proportion of shared area at 50% MCP	0.070	0.030	0.763
Abundance versus proportion of shared area at 50% MCP	0.190	0.936	0.388
Helminth richness versus proportion of shared area at 50% MCP	0.165	0.790	0.424
Number of overlaps and helminths infection in the wet season			
Prevalence versus number of overlaps at 50% MCP	NA		NA
Abundance versus number of overlaps at 50% MCP	NA		NA
Helminth richness versus number of overlaps at 50% MCP	NA		NA
Number of overlaps and helminths infection in the dry season			
Prevalence versus number of overlaps at 50% MCP	0.025	0.104	0.763
Abundance versus number of overlaps at 50% MCP	0.042	0.177	0.695
Helminth richness versus number of overlaps at 50% MCP	0.294	1.667	0.266

Key: NA - Not application: There was no variation in number of overlaps in the wet season

Table 5.4: Regression coefficient showing the relationship between parasite infection and home range size across baboon groups

Variable	r^2	F-statistics	*P*-value
Dry season			
Prevalence versus home range size at 50% MCP	0.340	2.065	0.224
Prevalence versus home range size at 100% MCP	0.758	12.53	**0.024**
Helminth richness versus home range size at 50% MCP	0.521	4.351	0.105
Helminth richness versus home range size at 100% MCP	0.954	83.81	**0.001**
Abundance versus home range size at 50% MCP	0.165	0.788	0.425
Abundance versus home range size at 100% MCP	0.366	2.311	0.203
Wet season			
Prevalence versus home range size at 50% MCP	0.246	1.303	0.317
Prevalence versus home range size at 100% MCP	0.102	0.455	0.537
Helminth richness versus home range size at 50% MCP	0.141	0.655	0.464
Helminth richness versus home range size at 100% MCP	0.092	0.405	0.559
Abundance versus home range size at 50% MCP	0.000	0.001	0.972
Abundance versus home range size at 100% MCP	0.029	0.120	0.746

Key: *p*-values in bold are statistically significant.

The association between home range and helminths infection was negative in the dry season whereby as home range size increases, it is predicted that helminths prevalence (Figure 5.1) and species richness declined (Figure 5.2). In the wet season, home range size did not have siginificant association with prevalence, abundance and helminths species richness (Table 5.4).

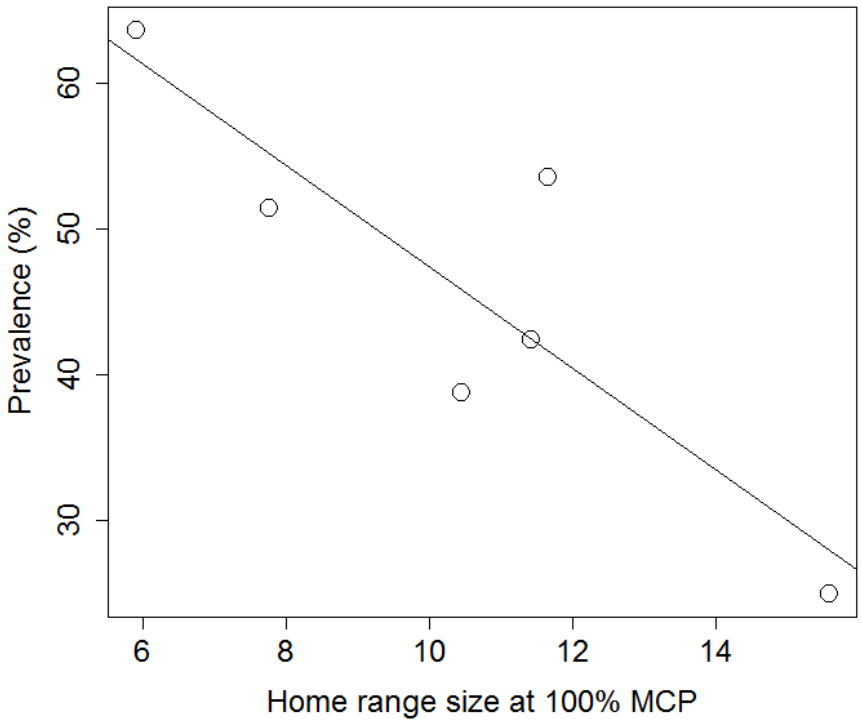


Figure 5.1: Negative association between prevalence and home range size (km^2) of baboon groups in Amboseli ecosystem, Kenya.

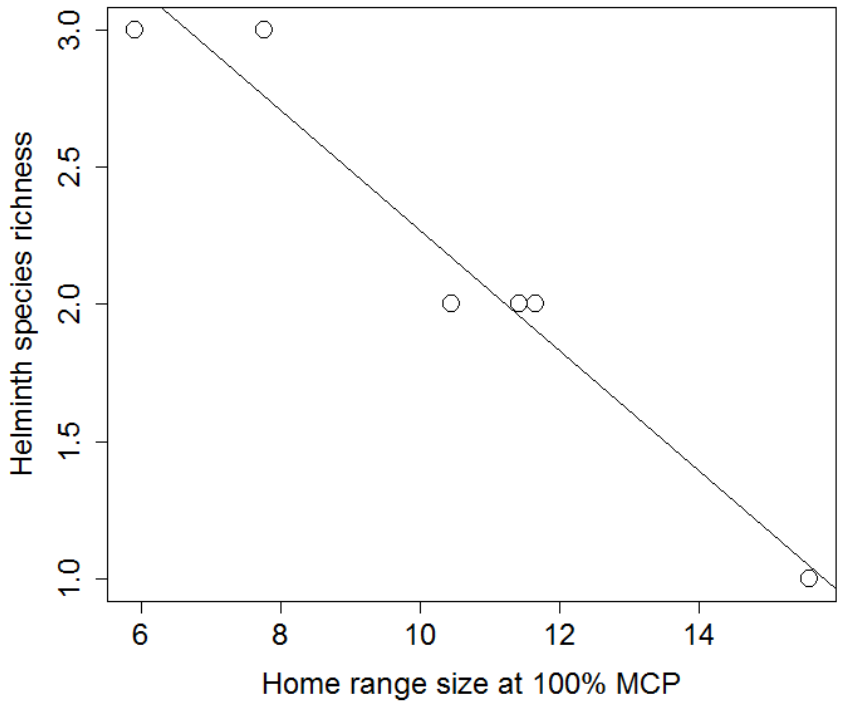


Figure 5.2: Negative association between helminth species richness and home range size (km^2) of baboon groups in Amboseli ecosystem, Kenya.

Results also indicated lack of significant association between mean prevalence and density of each baboon social group measured at 50% MCP (r_s = -0.086, n = 6, p = 0.919) or at 100% MCP (r_s = -0.086, n = 6, p = 0.919) levels of core habitat. Although baboon groups differed in the size of the group, there was no significant association between group size with either abundance (r_s = 0.486, n = 6, p = 0.356) or prevalence (r_s = -0.829, n = 6, p = 0.058).

There was no significant association between helminth species richness and group size (r_s = 0.253, n = 6, p = 0.309) or with density at 50% MCP (r_s = 0.001, n = 6, p = 0.950) and at 100% MCP (r_s = 0.215, n = 6, p = 0.354).

It was also observed that there was lack of significant association between helminth abundance and density of each baboon group measured at 100% MCP (r_s = 0.600, n = 6, p = 0.242) and at 50% MCP (r_s = 0.771, n = 6, p = 0.103) levels of core habitat. The association, though statistically insignificant, showed a positive trend in which high density is predictive of high helminth abundance (Figures 5.3 and 5.4).

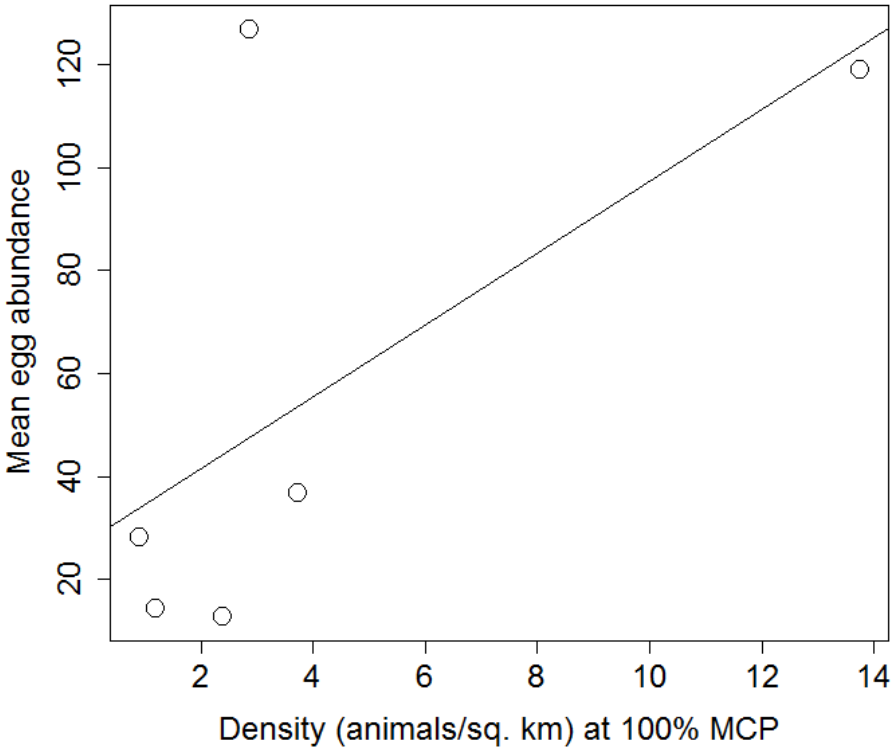


Figure 5.3: Association between mean egg abundance and density of baboon groups measured at 100% MCP level of core habitat.

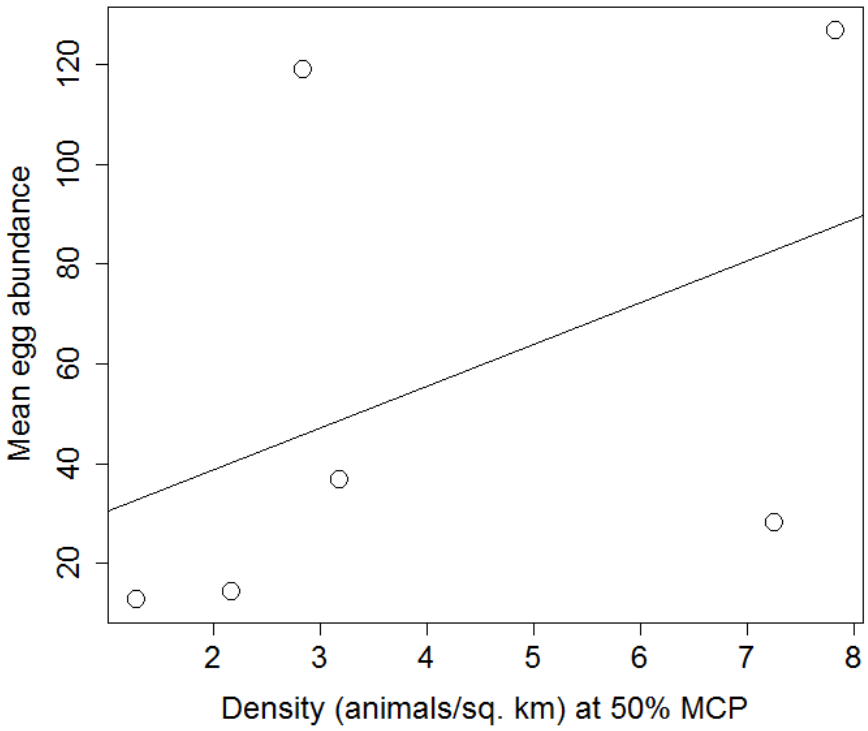


Figure 5.4: Association between mean egg abundance and density of baboon groups measured at 50% MCP level of core habitat

5.3.2 Effect of the degree of habitat overlap between baboon and alternative hosts on helminths infections in baboons

5.3.2.1 Dung pile count as index of overlap

Results showed that at 50% MCP of baboon home range, the degree of overlap (based on dung pile counts) between baboon and alternative hosts did not significantly influence either mean prevalence (r^2 = 0.441, t = -1.777, p = 0.150) or mean abundance (r^2 = 0.222, t = -1.068, p = 0.3458). Similarly at 100% MCP of home range, the degree of overlap did not significantly influence either mean abundance (r^2 = 0.452, t = -1.814, p = 0.144) or mean prevalence (r^2 = 0.006, t = 0.156, p = 0.884). There was no statistical association

between helminths species richness and dung pile counts at 50% MCP (r_s = 0.171, n = 6, t =0.85, p = 0.415) and 100% MCP (r_s = 0.001, n = 6, t = 0.05, p = 0.945).

5.3.2.2 Frequency of host sightings as index of overlap

There was statistical significance in the relationship between helminths species richness and host diversity at 50% MCP (r^2 = 0.655, $F_{(1,4)}$ = 7.594, p = 0.051) and at 100% MCP of habitat range (r^2 = 0.638, $F_{(1,4)}$ = 7.042, p = 0.057) only in the wet season (Table 5.5). The relationship between helminths richness and host diversity was inverse (Figure 5.5) whereby host diversity increases as helminth species richness declines. Similarly, the association between helminth species richness and Shannon-Wiener diversity index of alternative host species was statistically significant (r^2 = 0.727, $F_{(1,4)}$ = 10.64, p = 0.031). The relationship between helminths richness and Shannon-Wiener diversity index was also inversed (Figure 5.6) whereby Shannon-Wiener diversity index increases as helminths species richness declines. All other measures of degree of habitat overlap and helminths infection were not statistically significant (Table 5.5).

Table 5.5: Relations between degrees of habitat overlap based on frequency sightings between baboon and alternative hosts helminths infection in baboon

Dependent and independent variables	r^2	F-statistics	*P*- value
Parasite prevalence			
Host diversity in Dry season at 100% MCP	0.005	0.0195	0.896
Host diversity in Dry season at 50% MCP	0.050	0.209	0.671
Shannon-Wiener Index in Dry season at 100% MCP	0.114	0.517	0.512
Shannon-Wiener Index in Dry season at 50% MCP	0.700	0.3	0.613
Mean number of species sightings in Dry season at 100% MCP	0.150	0.706	0.448
Mean number of species sightings in Dry season at 50% MCP	0.167	0.804	0.421
Host diversity in wet season at 100% MCP	0.187	0.917	0.393
Host diversity in wet season at 50% MCP	0.267	1.46	0.294
Shannon-Wiener Index in wet season at 100% MCP	0.109	0.488	0.523
Shannon-Wiener Index in wet season at 50% MCP	0.217	1.107	0.352
Mean number of species sightings in wet season at 100% MCP	0.010	0.042	0.847
Mean number of species sightings in wet season at 50% MCP	0.143	0.667	0.460
Helminth species richness			
Host diversity in Dry season at 100% MCP	0.00	0.00	1.000
Host diversity in Dry season at 50% MCP	0.044	0.185	0.690
Shannon-Wiener Index in Dry season at 100% MCP	0.117	0.30	0.507
Shannon-Wiener Index in Dry season at 50% MCP	0.043	0.181	0.693
Mean number of species sightings in Dry season at 100% MCP	0.259	1.397	0.303
Mean number of species sightings in Dry season at 50% MCP	0.284	1.583	0.277
***Host diversity in wet season at 100% MCP**	***0.638***	***7.042***	***0.057***
***Host diversity in wet season at 50% MCP**	***0.655***	***7.594***	***0.051***

Shannon-Wiener Index in wet season at 100% MCP	0.000	0.000	0.998
***Shannon-Wiener Index in wet season at 50% MCP**	***0.727***	***10.64***	***0.031***
Mean number of species sightings in wet season at 100% MCP	0.012	0.049	0.836
Mean number of species sightings in wet season at 50% MCP	0.126	0.576	0.490
Helminth abundance			
Host diversity in Dry season at 100% MCP	0.051	0.216	0.666
Host diversity in Dry season at 50% MCP	0.389	2.548	0.186
Shannon-Wiener Index in Dry season at 100% MCP	0.000	0.000	0.991
Shannon-Wiener Index in Dry season at 50% MCP	0.526	4.438	0.103
Mean number of species sightings in Dry season at 100% MCP	0.300	1.717	0.260
Mean number of species sightings in Dry season at 50% MCP	0.354	2.193	0.213
Host diversity in wet season at 100% MCP	0.042	0.175	0.697
Host diversity in wet season at 50% MCP	0.069	0.299	0.614
Shannon-Wiener Index in wet season at 100% MCP	0.323	1.905	0.240
Shannon-Wiener Index in wet season at 50% MCP	0.005	0.022	0.890
Mean number of species sightings in wet season at 100% MCP	0.000	0.000	0.989
Mean number of species sightings in wet season at 50% MCP	0.001	0.003	0.959

Key: *Bold and italicized values indicate statistical significance

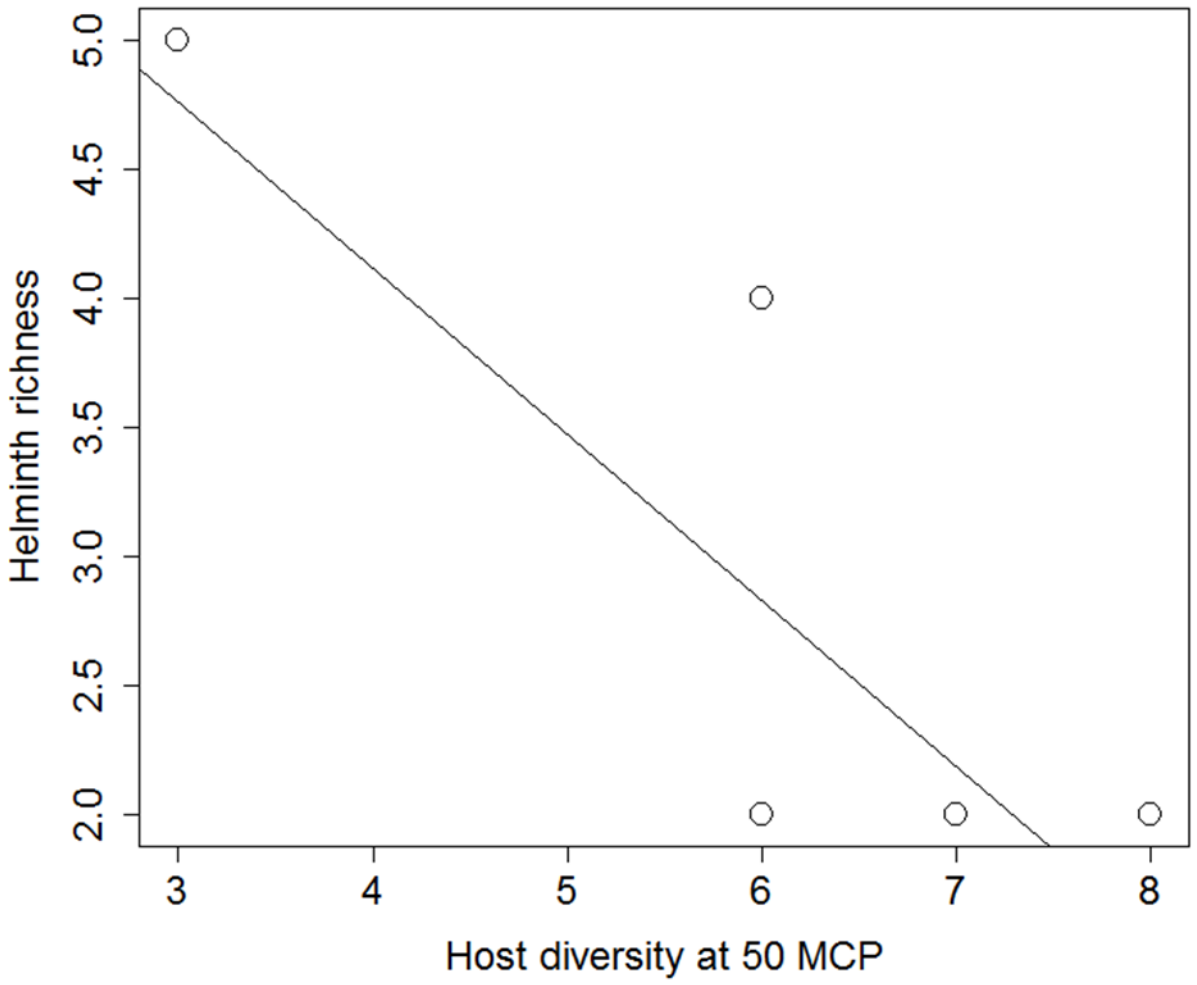


Figure 5.5: Inverse relationship between helminth richness and host diversity in a baboon group's habitat range measured at 50% MCP.

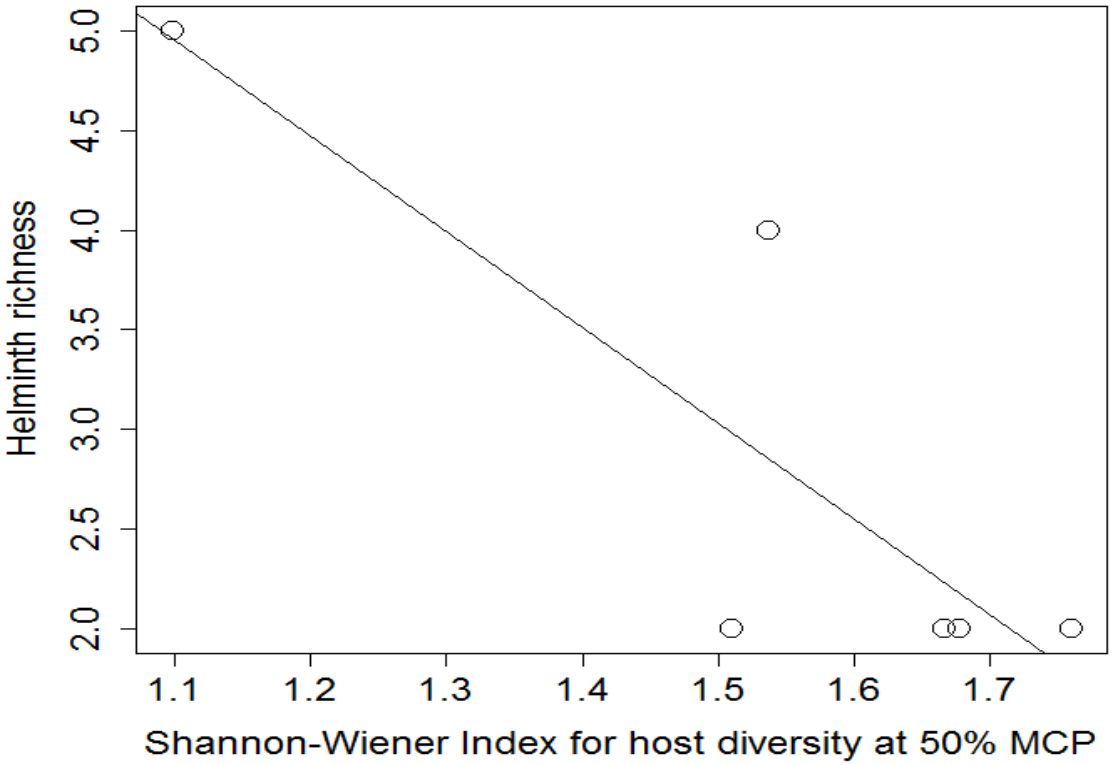


Figure 5.6: Inverse relationship between helminth richness and Shannon-Wiener index for host diversity in a baboon's habitat range measured at 50% MCP.

5.4. Discussion

5.4.1: Effects of habitat overlap between baboon groups on helminth infections

The effect of home range overlap on prevalence of helminths was evaluated across baboon groups and results demonstrated that despite the close geographical proximity and even high degree of home range overlaps, prevalence were significantly different across baboon groups and also seasonally. Factors driving this kind of pattern are not obvious but may be linked to prevailing heterogeneities in group composition in terms of age, sex, immune status, coinfections and reproductive status, factors known to influence intra-species variation in helminth prevalence (MacIntosh *et al*., 2010; Brown and Symondson, 2014). For instance, prevalence in helminths is always male-biased across mammalian taxa (Poulin, 1996; Moore and Wilson, 2002), which means that a baboon group comprising of more male than female individuals is likely to experience higher prevalence. Inter-troop difference in helminth prevalence in baboon population seems not to be unique for the Amboseli baboon. Ravasi, (2009) observed that helminth prevalence varied among contiguous Chacma baboon troops in a South African population. In the present study, it was remarkable that the inter-group variations in helminth prevalence occured at a very fine spatial scale of proximity, which may imply social structuring towards infection. Such socially driven difference in prevalence are rare in mammalian community (Gonzalez-Hernandez *et al*., 2011) but have been demonstrated by household clustering in helminths prevalence in humans living in close proximity (Chan *et al*., 1994; Walker *et al*., 2011). Human infections with *Ascaris lumbricoides* tend to display individual predisposition and household clustering (Walker *et al*., 2011). The cause of such patterns are incompletely understood but suspected to be

contributed by heterogeneity in exposure, genetic- and immunologically-mediated susceptibilitity (Holland, 2009). Inference based on these occurrence imply that social clustering of infection in social host communities is most likely hinged on heterogeneity of transmission contacts across social groups (Woolhouse *et al*., 2007; Lloyd-Smith *et al*., 2005).

This study did not find significant association between helminth prevalence, abundance and species richness with degree of habitat overlap between baboon groups. This means that baboon groups that shared a higher proportion of their home range with other groups were not more infected compared to groups with less home range sharing (Table 5.3). The lack of association between degree of overlap and helminths infection rates implies that among conspecifics, maximum overlap of home range or presence of exclusive portions of habitat has no significant effect on transmission rates. Similarly, a baboon group that shared their home range with more social groups was not highly infected than groups whose home ranges were shared by few groups. These finding are in agreement with that of Gonzalez-Hernandez *et al*., (2011) in Howler monkeys, which may mean that transmission rate is unaffected by increased overlap or immigration of conspecifics.

The relationship between size of home range with prevalence (Figure 5.1) and helminth species richness (Figure 5.2), were inverse and statistically significant which suggests that as size of home range increases, helminth prevalence and species richness decline. These relationships imply that baboon groups with larger home ranges have less prevalence and helminth species richness. It is generally expected that animals which occupy large home ranges or range widely should have higher prevalence and parasite species richness because they will likely encounter greater diversity of habitats and host

taxa, which predispose them to higher risks of infection with more diverse parasites (Bordes *et al*., 2009). However, most of the previous studies contradict such a notion, and agree with findings from this study that demonstrates a negative association between home range size and helminth species richness. Negative association between size of home range and parasite species richness have been demonstrated among primates (Nunn *et al*., 2003), carnivores (Lindenfors *et al*., 2007), rodents and lagomorphs (Bordes *et al*., 2009) whereas lack of association was observed in ungulates (Ezenwa *et al*., 2006). This means that factors that drive parasite species richness across host species are inconclusive even though the present study suggests home range size is a determinant. Home range size is one of the three universal predictors of parasite species richness suggested along side host body size and population density (Kamiya *et al*., 2014).

Bordes *et al*., (2009) posits that low prevalence and parasite species richness in animals that range widely and eventually occupy vast home ranges may be a strategy to avoid or limit infection. Such strategy is based on the concept of migratory escape whereby animals move away from parasite contaminated regions of their habitat. Post-calving migration of Reindeers is viewed as migratory escape from regions infested with warble fly (Folstad *et al*., 1991). Similarly, the frequent change of sleeping groves by baboons is also explained as behavioral strategy to avoid helminth contaminated areas (Hausfater and Meade, 1982). This theory was developed while investigating patterns of helminth infection in Amboseli baboons, whereby Hausfater and Meade, (1982) confirmed that the soil from beneath sleeping groves not used by baboons yielded only 74.8 nematodes per 100 g while soil from open areas adjacent to groves, yielded no larvae or adult nematodes at all. Further in their study, Hausfater and Meade, (1982) observed that soil sample (wet

weight, 1146.5 g) collected under known sleeping groves yielded 4,620 larvae and adult nematodes, which was on average 402.9 nematodes per 100 g of soil.

In the present study, there was no correlation between group size and density of the baboon groups with helminth prevalence. Neither was there correlation between group size and density with abundance. Group-living is one ecological factor that enhances contact rates and disease transmission, which means that group-living is likely to predict infection risk for fecally-transmitted parasites (Davies *et al*., 1991; Côté and Poulin, 1995; Morand and Poulin, 1998; Arneberg, 2002). The association between group size and density with helminth infection rates is based on the epidemiological model that predicts positive correlation between host density and parasite prevalence, abundance and species richness (Arneberg, 2002; Nunn *et al*., 2003). This model has been further supported by a meta-analysis that demonstrates the tendency for group size to positively correlate with both prevalence and abundance for directly- and indirectly-transmitted parasites (Patterson and Ruckstuhl, 2013). Lack of association between group size and helminth abundance, particularly cestodes have been demonstrated in mole rats (Viljoen *et al*., 2011). This suggests that the association between group size and density with helminths infection rates displays a continuum of relationship that include absence, positive and negative which could imply that there are underlying factors that influence the relationship. For instance, Moore *et al*., (1988) clarifies that positive relationship between group size and helminths infection rates should be predicted only when the involved parasites have rapid, monoxenous life cycles and when the host social groups are stable. In the present study, the Amboseli baboons harboured several heteroexenous species of helminths such as *Primasubulura, Streptopharagus, Spirurina*, but also

harboured monoexenous helminths species such as the strongylids, *Strongyloides* spp. and *Trichuris* spp.

5.4.2 Effects of habitat overlap between baboons and alternative hosts on helminth infections in baboons.

The degree of habitat overlap based on dung pile counts did not show significant association with helminth prevalence, abundance or species richness in the baboon population. However, degree of habitat overlap based on frequency of sightings showed significant association between helminths species richness with both host diversity and Shannon-Wiener diversity index. This means that as host diversity increases in a home range of baboons, it is predicted that they will harbor less helminths species richness. Dung pile count and frequency of observation differed in their association with helminths species richness. It seems that frequency of host observation is a better index for host diversity rather than being based on standing crop of dung pile. This is because standing crop is subject to environmental and climatic variables which may misrepresent the actual host diversity.

Previous study by Ezenwa, (2003) did not find any association between host diversity and helminth species richness. As such, Ezenwa, (2003) postulated that cross-species host interactions may not be a key predictor of parasite richness across taxa. Similarly, Watve and Sukumar, (1995) did not find any association between helminth species richness and across diversity of 12 mammalian hosts. Although Ezenwa, (2003), fronts a lack of association between host diversity and helminths species richness, there are also two divergent theories; the first theory suggests increased host diversity dampens helminths species richness while the second theory suggests increased host diversity escalates

helminths species richness (Keesing *et al*., 2006; Ostfeld and Keesing, 2012; Johnson *et al*., 2013; Wodjak *et al*., 2014). Results in the present study are consistent with the theory that increased host diversity dampens helminths species richness which is explained as the 'dilution effect' on helminth transmission. The 'dilution effect' is a product of increased host diversity coupled by variations in host competence (Ostfeld and Keesing, 2012) whereby enhanced encounter rates between hosts and infective stages lead to reduced transmission (Keesing *et al*., 2006). This is because non-competent hosts absorb infective stages without getting infected hence reduces availability of infective stages for competent hosts. For instance, increase in wildlife host diversity has been reported to reduce the transmission of the tick-borne *Borrelia burgdorferi,* potentially decreasing the risk of Lyme disease in humans (Ostfeld and Keesing, 2012).

The contrasting theory predicts positive correlations between host and parasite species richness (Hechinger and Lafferty, 2005). Host rich habitats (areas with high degree of habitat overlap) are expected to have increased parasite infection, a perspective hypothesized as 'host diversity begets parasite diversity' (Hechinger and Lafferty, 2005). This is because rich diversity of hosts offers opportunity for parasite colonization (Johnson *et al*., 2013). On the baseline of the two divergent views is that host species composition, density of each host species and host competence are traits that are critical for transmission and yet they vary dramatically in a natural host community (Wojdak *et al*., 2014). In summary, baboon groups whose habitat was occupied by high host diversity (increased degree of overlap with heterospecifics) harboured less helminths diversity. Thus, in this study, the first theory suffices.

CHAPTER SIX: GENETIC IDENTIFICATION OF NEMATODES FROM SYMPATRIC BABOONS, VERVET MONKEYS AND UNGULATES IN AMBOSELI ECOSYSTEM, KENYA

6.1 Introduction

Epidemiology of nematodes in both livestock and wildlife in Kenya is not well understood, yet wildlife and livestock harbor and share numerous species. Wild mammalian hosts play a central role in shaping the epidemiology of helminths between wildlife and livestock. For instance, migratory Saiga antelopes have been observed to act as vectors of specific nematodes between two disparate herds of sheep in Kazakhstan (Morgan *et al*., 2007). The interface between wildlife and livestock in Kenya is getting more intimate which creates an opportunity for cross-species transmission.

Since the helminth species in most wild mammalin hosts are not well known and yet they are likely to spill-over to livestock, it is plausible to use the most sensitive and specific tools of helminth identification. Molecular tools are therefore useful not only in detecting previously unknown helminths species but can also detect subtle genotypic changes that might have occurred in helminth species shared across multiple hosts (Akkari *et al*., 2013).

Although there are numerous information on the diversity and prevalence of helminths in various species of domestic animals in Kenya (Nga'ng'a *et al*., 2004; Ng'ang'a *et al*., 2006; Nginyi *et al*., 2001), this study will specifically add information on the epidemiology of nematodes in free-grazing livestock and wild ungulates as well as non-human primates. Further, as much as wildlife-livestock interaction is widespread in Kenya, this will be one of the few studies to investigate helminths at the interface

(Ezenwa, 2003: Vanderwaal *et al*., 2014). However, it is only the study by Vanderwaal *et al*., (2014) that has investigated helminths of a mixed livestock-wildlife host system in Kenya.

This study did not only determine helminth species of a mixed wildlife-livestock system in Kenya but also applied genetic techniques, which are considered to be of higher sensitivity and specificity for differentiating closely related nematodes (Zarlenga *et al*., 1998; 1999; Gasser *et al*., 2008). The genetic procedure was based on two genetic markers, the Internal transcribed spacer (ITS) region of ribosomal DNA (rDNA) and a region of the mitochondrial cytochrome oxidase 1 to differentiate closely related nematodes and intra-specific strains as well as discovery of cryptic species (Zarlenga *et al*., 1999; Eysker and Ploeger, 2000; Archie and Ezenwa, 2011; Ghai *et al*., 2014). More interestingly, molecular techniques offer opportunity to understand how generalist nematodes such as *Oesophagostomum bifurcum* or *Trichostrongylus axei* distribute themselves among sympatric hosts. These markers were applied on nematode larvae extracted from faecal cultures of the animals.

6.2 Material and Methods

6.2.1 Fecal cultures

Dung samples were collected in the study area as described in Chapter 3.2.1. The sources and collection of faecal material is given in previous chapters. 10 containers of pooled fresh dung were collected from each animal species in the dry and wet seasons.

The culture procedure was performed as described by Archie and Ezenwa, (2011). Pooled fecal sample from each host species was moistened with water and gently mixed to form a paste. Faecal cultures were prepared by scooping approximately 10g of the fecal paste and placing them in to culture jars which were left at room temperature for 12 days. The fecal cultures were checked daily, moistened with water and gently stirred to prevent fungal growth.

6.2.2 Larval extraction from faecal cultures

Larval extraction from faecal cultures as described by Archie and Ezenwa, (2011) was carried out from the 13th day; specifically, the culture jars with fecal material were filled with lukewarm tap water, stirred and inverted on a glass petri dish. The exposed area around the inverted jar was filled with clean lukewarm water and left to stand for 12 hours at room temperature. The larvae migrated from the murky fecal mixture in the inverted jar to the clean water on the exposed part of the petri dish. The larval suspension (water containing the larvae) was pipetted out into an extra clean petri dish and examined under a stereo-microscope at 10 - 40X magnification. When a larva was observed, it was picked using a pipette fitted with pipette tip (pointed tip sliced off), and transferred into a labeled cryovial containing 200µl of absolute ethanol.

However, while examining larvae under the sterio-microscope, sluggish-moving larvae were observed and were picked and examined further using higher magnification under a digital compound microscope. The examined life-stage of the worm was peculiar in that it had both both eggs and live larvae in the uterus, which is a viviparous trait. A total of 10 individual worms were separated and each larva was preserved separately in its cryovial and was labeled "viviparous nematode" to facilitate matching with sequences after genetic analysis. The identity of the host species from which faecal samples for culture were collected was also labeled on the cryovials. All the the larvae were maintained cool at 4^0C until processing.

6.2.3 DNA extraction

DNA was extracted from 977 larvae/worms as described by Archie and Ezenwa, (2011). Briefly, ethanol that was used to preserve the larvae was first evaporated out through vacuum centrifugation. Once the tubes were dry, 5 µl of PCR-quality water and 15µl of lysis buffer [2 µl 10X PCR Gold buffer (Fisher Scientific, New Jersey, USA); 0.8 µl Magnesium Chloride (Applied Biosystems, USA); 2 µl of 4.5% Nonidet P-40 (Amresco ®, Ohio, USA); 2 µl of 4.5% Tween 20 (Fisher Scientific, New Jersey, USA); 2 µl of a 2mg/ml proteinase K (Applied Biosystems, USA) and 6.2 µl MilliQ water] was added to each tube. The tube was centrifuged briefly to bring the liquid down and the sample was placed at -80^0C for at least 20 minutes, ensuring that the liquid in the tube had frozen completely. The sample was removed from the freezer and placed directly on heat block at 60^0C for 100 minutes followed immediately by 20 minutes at 94^0C. This procedure was to break the cuticle of the larvae and expose DNA via thermal shock. The tubes were removed from the heat block and centrifuged briefly to remove drops from the lid. The

DNA extracts were diluted 3x by adding 40ul of PCR-quality water and stored at -20^{0}C until use.

6.2.4 Polymerase Chain Reaction (PCR)

PCR was undertaken as previously described by McLean *et al*., (2012). Each DNA extract was amplified at two loci; first by the internal transcribed spacer region of ribosomal DNA (rDNA) that spans ITS1, 5.8S and ITS2 and second by a portion of the cytochrome C oxidase 1 gene of the mitochondrial DNA (mtDNA). The ITS rDNA locus was selected because it is reliable in differentiating among closely related species of nematodes (Chilton *et al*., 1995; Hung *et al*., 1996; Newton *et al*., 1998; Gasser and Newton, 2000; Blouin, 2002; Gasser *et al*., 2008; McLean *et al*., 2012). The ITS marker is also commonly used due to its lower level of intra-species polymorphism compared to mtDNA (Denver *et al*., 2000; Blouin, 2002). The mtDNA was also used because of its relatively higher degree of variability that makes it a good choice for differentiating cryptic species (Blouin, 2002).

The ITS region was amplified by two primers, first by NC5 (forward, 5'-GTAGGTGAACCTGCGGAAGGATCATT-3') and NC2 (reverse, 5'-TTAGTTTCTTTTCCTCCGCT-3'). Amplification (Eppendorf Mastercycler® Pro, USA) was carried out in 15 µl reactions containing 1.5 µl of genomic DNA, 6 µl of 5 PRIME hotmaster mix (Hamburg, Germany), 0.75 µl of each primer (10 µM) and 6 µl of PCR-quality water. Amplification was preceded by a 2 minute polymerase activation step at 90^{0}C, followed by 39 cycles of 45 sec each at 57^{0}C annealing, 72^{0}C extension and 95^{0}C denaturation. Amplification was terminated by a final extension step at 72^{0}C for 5

minutes. The second ITS primer used was NC1 (forward, 5'-ACGTCTGGTTCAGGGTTGTT-3') and NC2 (reverse, 5'-TTAGTTTCTTTTCCTCCGCT-3'). The PCR condition for this primer was identical to the (NC5-NC2 primer) conditions except that its annealing temperature was lower at 55^0C (McLean *et al*., 2012).

The mtDNA was amplified using primer LCO1490 (forward, 5'-GGTCAACAAATCATAAAGATATTGG-3') and HCO2198 (reverse, 5'-TAAACTTCAGGGTGACCAAAAAATCA-3') (Folmer *et al*., 1994). Amplification was carried out in 15 µl reactions containing 1.5 µl of genomic DNA, 6 µl of 5 PRIME hotmaster mix (Hamburg, Germany), 0.75 µl of each primer (10 µM) and 6 µl of PCR-quality water. Amplification was preceded by a 2 minute polymerase activation step at 90^0C, followed 39 cycles of 1-min each at 42^0C annealing, 72^0C extension and 95^0C denaturation. Amplification was terminated by a final extension step at 72^0C for 5 minutes. Gel electrophoresis (1.5% Agar, GP2 MIDSCI™, USA) was used to detect amplifications.

6.2.5 Sequencing and sequence analysis

Positive products were purified by ExoSAP-IT™ and submitted for sequencing at the Genomics unit of the University of Washington, Seattle, Washington, USA. The products were sequenced in both directions using Dye Terminator sequencer (Applied biosystems). Sequence data were examined and edited using Sequencher™ software v. 4.10 (Gene codes corporation, Ann Arbor, Michigan USA) whereby only 121 (quality of each strand >70%) and complimentary contiguous sequences >85% in quality, were

considered clean. The cleaned sequences for each host were, cattle (n = 10), goat (n = 10), Grant gazelle (n = 5), baboon (n = 27), impala (n = 14), Thomson's gazelle (n = 13), Vervet monkey (n = 42).

These sequences were searched for their closest match in the Genebank using Basic Local Alignment Search Tool (BLAST) of the National Center for Biotechnology Information (NCBI).

6.2.6 Phylogenetic analysis

All sequences were aligned using ClustalX2 software with alignment parameters (gap opening penalty at 15; gap extension at 6.66 and delay divergence set at 30%). To determine the model that best-fit nucleotide substitution, MEGA 5.0 software parameters were set to include all sites, and branch swap set as 'very strong' while all the 1st, 2nd, and 3rd non-coding positions were all used. Model with the lowest Bayesian Information Criterion (BIC) scores was considered to best describe the substitution patterns.

Therefore the model that suited phylogenetic reconstruction of mitochondrial gene sequences of nematodes in the sub-family Cyathostominae was the Tamura 3-parameter with Gamma distribution (T92+G) model whereas for the ITS gene, the Tamura 3-parameter with invariant sites (T92+I) was the best fit model. The model that suited phylogenetic reconstruction of ITS gene sequences of Trichostrongylid nematodes was also the T92+G model. Phylogenetic reconstruction for *S. stercoralis* and *S.fuelleborni* involved a mix of ITS and mtDNA sequences (McDonnell *et al*., 2000) and modeled by the Tamura Nei (TN3). All phylogenetic trees were derived by MEGA 5.0 based on

maximum likelihood method at bootstraps of 1000 replicates. Sequences from Genebank are summarized in Tables 6.1 – 6.4

Table 6.1: Reference sequences of mtDNA selected from genebank that were included in the phylogenetic tree

Nematode name	**Host**	**Country**	**Gene**	**Accession number**
Khalilia sameera	Elephant	Kenya	mtDNA	JN252508.1
Murshidia linstowi	Elephant	Kenya	mtDNA	JN252637.1
Murshidia africana	Elephant	Kenya	mtDNA	JN252515.1
Murshidia longicaudata	Elephant	Kenya	mtDNA	JN252539.1
Quilonia africana	Elephant	Kenya	mtDNA	JN252509.1
Strongylus vulgaris	-	USA	mtDNA	U57040.1
Triodontophorus serratus	Horse	UK	mtDNA	AF263488.1
Cylicocyclus nassatus	Horse	Ukraine	mtDNA	EU753193.1
Poteriostomum imperidentatum	Horse	UK	mtDNA	AF263483.1
Coronocyclus coronatus	Horse	UK	mtDNA	AF263473.1
Cylicostephanus minutus	Horse	UK	mtDNA	AF263479.1
Cyathostomum catinatum	Horse	UK	mtDNA	AF263472.1
Tridentoinfundibulum gobi	Horse	UK	mtDNA	AF263476.1

Table 6.2: Reference sequences of ITS rDNA from Genebank that was included in the phylogenetic tree.

Nematode name	Host	Country	Gene	Accession number
Kiluluma solitaria	White rhino	Australia	ITS	JX982337
Kiluluma ceratotherii	White rhino	Australia	ITS	JX982335
Kiluluma sp.	White rhino	Australia	ITS	JX982336
Khalilia sameera	Elephant	Kenya	ITS	JN252688
Murshidia linstowi	Elephant	Kenya	ITS	JN252662
Murshidia africana	Elephant	Kenya	ITS	JN252687
Murshidia longicaudata	Elephant	Kenya	ITS	JN252686)
Quilonia africana	Elephant	Kenya	ITS	JN252693
Strongylus equinus	Equine	Australia	ITS	AJ228250
Cylicocyclus insigne	Horse	Germany	ITS	AF447759
Cylicocyclus nassatus	Donkey	China	ITS	JQ906422
Cylicocyclus radiatus	Donkey	China	ITS	JQ906423
Cylicocyclus elongatus	Donkey	China	ITS	JQ906417
Cylicocyclus auriculatus	Donkey	China	ITS	JQ906416
Cyathostomum labratum	Equine	Australia	ITS	AJ004854
Cylicocyclus ashworthi	Donkey	China	ITS	JQ906412
Cylicocyclus adersi	Donkey	China	ITS	JQ906411
Coronocyclus coronatus	Donkey	China	ITS	JN786951

Table 6.3: References for sequences of Trichostrongylids selected from Genebank and included in the phylogenetic tree

Nematode name	Host	Country	Gene	Accession number
Oesophagostomum bifurcum	Mona monkey	Australia	ITS	AF136575
Oesophagostomum bifurcum	Human	-	ITS	Y11733
Oesophagostomum bifurcum	*Macaca*	China	ITS	KF319024
Oesophagostomum stephanostomum	Gorilla	Gabon	ITS	AB821022
Trichostrongylus colubriformis	Sheep	New Zealand	ITS	KC998744
Trichostrongylus colubriformis	Human	Thailand	ITS	KC337067
Trichostrongylus axei	Sheep	Russia	ITS	EF427622
Teladorsagia circumcincta	Sheep	New Zealand	ITS	KC998708
Teladorsagia circumcincta	Sheep	Australia	ITS	X86026
Cooperia oncophora	Sheep	Australia	ITS	X83561
Cooperia surnabada	Sheep	Australia	ITS	AJ000032
Cooperia punctata	Cattle	New Zealand	ITS	KC998744
Cooperia punctata	Sheep	Australia	ITS	X83560
Haemonchus contortus	Goat	Tunisia	ITS	JX901146
Haemonchus contortus	Sheep	Iran	ITS	HQ389229
Haemonchus contortus	Giraffe	USA	ITS	EU084689
Strongylus edentates	Equine	Australia	ITS	X77807

Table 6.4: References for sequences of species of Strongyloides and Necator species selected from the Genebank and included in the phylogenetic tree

Nematode name	Host	Country	Gene	Accession number
Strongyloides stercoralis	-	Australia	ITS	JX489154
Strongyloides stercoralis	-	Iran	ITS	EF545004
Strongyloides stercoralis	Orangutans	Indonesia	ITS	JF699149
Strongyloides stercoralis	Dog	USA	ITS	U43962
Strongyloides fuelleborni	Human	TZ/Japan	mtDNA	AB526282
Strongyloides fuelleborni	Macaca	Japan	mtDNA	AB526291
Strongyloides fuelleborni	Macaca	Japan	mtDNA	AB526293
Strongyloides fuelleborni	Macaca	Japan	mtDNA	AB677957
Strongyloides fuelleborni	Macaca	Japan	mtDNA	AB526290
Strongyloides fuelleborni	Gorilla	Gabon	mtDNA	AB526289
Strongyloides fuelleborni	Chimpanzee	Gabon	mtDNA	AB526288
Strongyloides fuelleborni	Baboon	Tanzania	mtDNA	AB526285
Strongyloides fuelleborni	Baboon	Tanzania	mtDNA	AB526306
Necator sp	Human	China	mtDNA	AJ417719

6.3 Results

6.3.1: Sequence analysis

A total of 977 larvae were extracted from the faecal cultures of the different hosts. DNA was extracted from all the larvae, however after amplification, only 285 yielded positive amplicons for sequencing. The 285 amplicons were sequenced and only 121 sequences were of good quality for further editing and analysis. After sequence cleaning and editing, only 67 sequences matched with nematodes in the Genebank (Table 6.1). The rest of the sequences were discarded because they matched with organisms that were not nematodes, which may be due to contamination. The 67 cleaned sequences accounted for 6.8% only out of the total larvae/worms that were initially processed. Of these sequences, the highest proportion (83.6%) 56/67 was based on mitochondrial gene while 16.4% (11/67) were based on ITS gene of the ribosomal DNA. Most of the sequences (n = 49) were identified as nematodes that belong to the sub-family Cyathostominae, hereafter referred to as *Cyathostominae* nematodes (Table 6.5). The rest of the identified helminth species are listed in Table 6.5.

6.3.2 Genetic analysis

The genetic sequences of both MtDNA and ITSrDNA of the *Cyathostominae* nematodes showed different evolutionary patterns (Figure 6.1 and Figure 6.2). Evolutionary pattern based on MtDNA sequences of *Cyathostominae* nematodes revealed that the nematodes separated into three clades of which one comprised of nine isolates identified from baboon (Figure 6.1). Another clade consisted of three baboon isolates and species of *Murshidia, Quilonia* and *Khalilia*, which usually infect elephants and rhinoceros (Figure 6.1).

Table 6.5: Identified nematodes by the two gene markers.

Identified helminths	Host Animal	Gene markers		
		MtDNA gene	ITS gene	Total
Cyathostominae nematodes	Baboon	13	2	15
	Vervet monkey	20	2	22
	Impala	0	1	1
	Thomson's gazelle	4	0	4
	Cattle	4	0	4
	Goat	3	0	3
Strongyloides stercoralis	Baboon	0	7	7
Strongyloides fuelleborni	Baboon	5	0	5
Ooesophagostomum bifurcum	Baboon	0	1	1
Trichostrongylus colubriformis	Baboon	0	1	1
Haemonchus contortus	Goat	0	1	1
Cooperia oncophora	Cattle	0	2	2
Teladosargia circumcincta	Grant's gazelle	0	1	1
Total		**49**	**18**	**67**

The other clade comprised of 44 sequence haplotypes with majority identified from vervet monkey (*n* = 20) while the rest were from Thomson's gazelle (*n* =4), cattle (*n* = 4), goat (*n* = 3) and baboon (*n* = 1). In the evolutionary tree, the equine cyathostominae, which included species such as *Coronocyclus, Cylicostephanus* and *Cyathostomum*, clustered in a separate clade. The helminth that served as an out-group for the evolutionary relationship was *Strongylus vulgaris*, the equine 'large cyathostominae'.

The evolutionary pattern based on ITS gene sequences of *Cyathostominae* nematode revealed that the nematodes clustered into a single (mixed host) clade (Figure 6.2). The *Cyathostominae* nematodes that clustered in the clade were identified from vervet monkey (*n* = 2), baboon (*n* = 2) and impala (*n* = 1). In addition, a nematode identified from baboon and named as *Baboon_KeH22* (Figure 6.1) and *Baboon_KEH22a* (Figure 6.2) was the only nematode identified by mitochondrial and ITS genes, respectively.

Nematodes usually harbored by elephants and rhinoceros separated into their own clade apart from clades comprising nematodes usually harbored by equines (Figure 6.2). The helminth that served as an out-group for the evolutionary relationship was *Strongylus equinus*, the equine 'large cyathostominae'

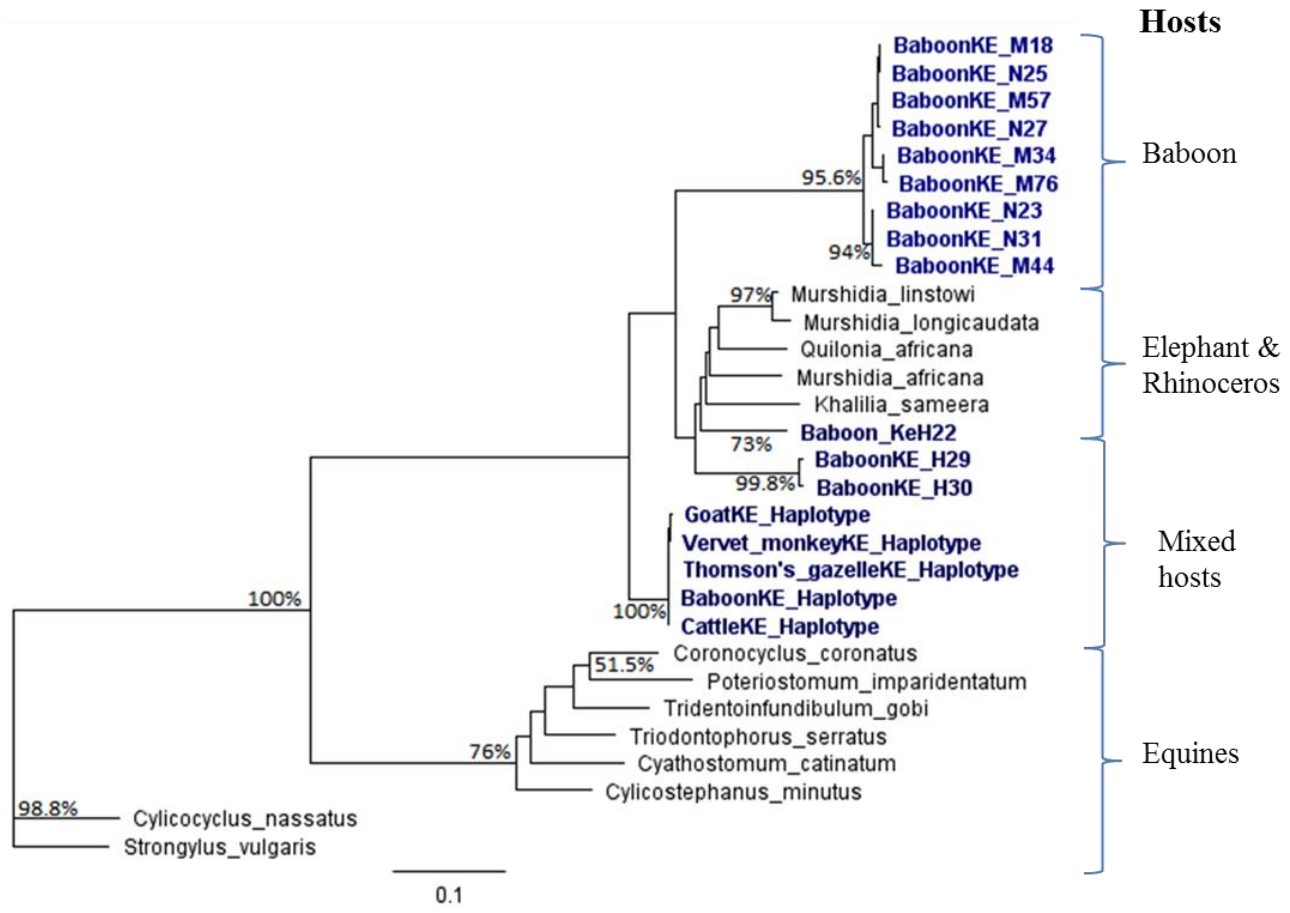


Figure 6.1: Evolutionary relationships of the present isolates (blue bold) to strongylid nematodes of the sub-family Cyathostominae selected from the genebank. The rooted maximum likelihood tree based on mtDNA was derived at 1000 bootstrap replicates using *Strongylus vulgaris* as outgroup. The numbers next to branches represent bootstrap values where values <50% were collapsed.

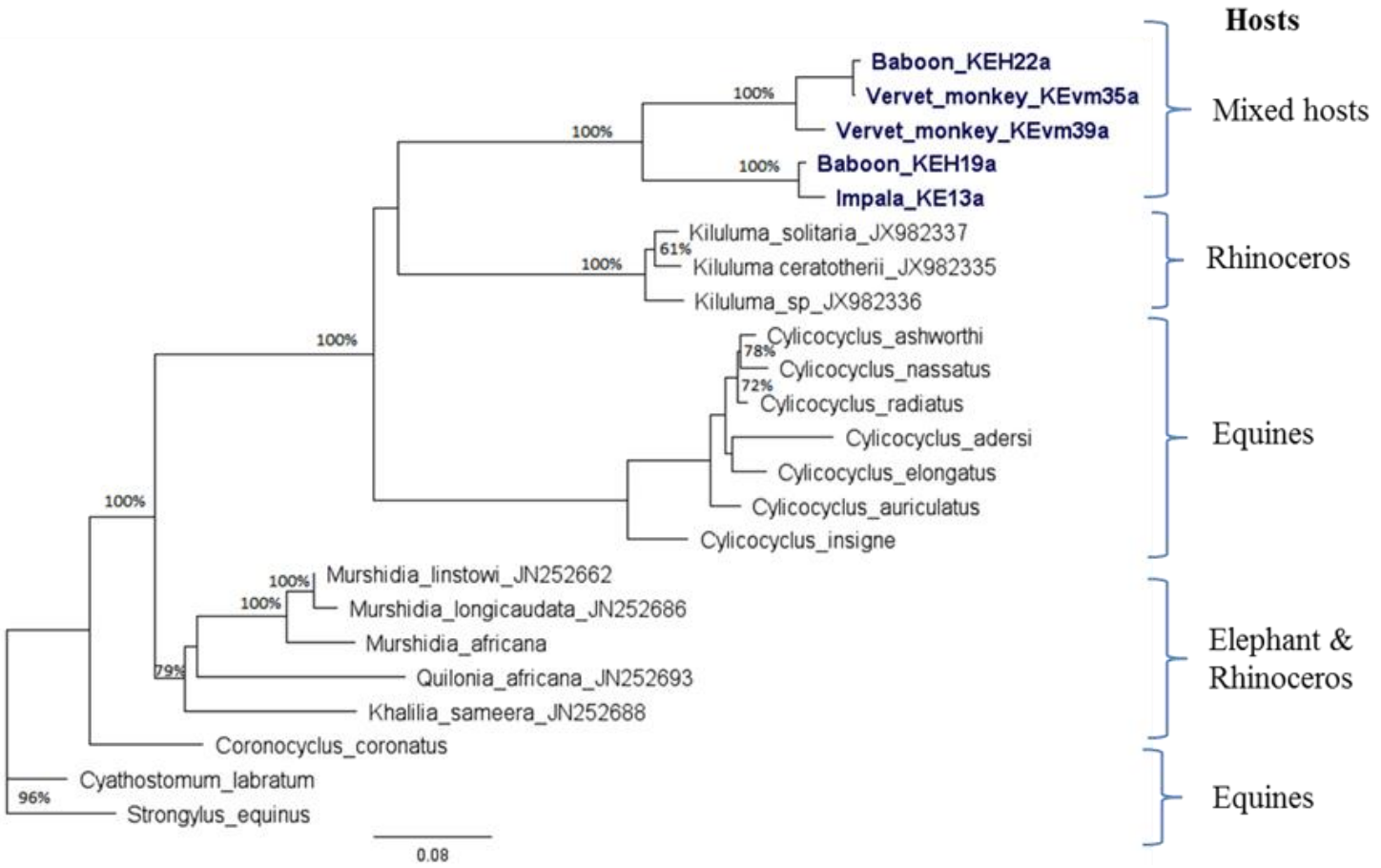


Figure 6.2: Evolutionary relationships of the present isolates (blue bold) to strongylid nematodes of the sub-family Cyathostominae selected from the genebank. The rooted maximum likelihood tree based on internal transcribed spacers (ITS) of the ribosomal was derived at 1000 bootstrap replicates using *Strongylus vulgaris* as outgroup. The numbers next to branches represent bootstrap values where values <50% were collapsed.

The two species of *Strongyloides* were identified from baboons and their evolutionary relationship revealed apparent different rates of evolution (Figure 6.3). The *S. fuelleborni* from Kenyan baboons clustered into a single clade that was monophyletic with *S. fuelleborni* identified from various hosts in Tanzania, Gabon and Japan (Figure 6.3). The evolutionary relationship of *S. fuelleborni* identified from different hosts demonstrates genetic sub-structuring driven by geographical location rather than by host species (Figure 6.3). Moreover, *S. fuelleborni* identified from different geographical locations in Africa reveal uniform rates of evolution that is divergent from those in Japan. In contrast, *S. fuelleborni* identified from the same host species but inhabiting different regions in Japan demonstrate incongruent rates of evolution as those in Yaku Island are basal to those in other Japanese regions (Figure 6.3).

Strongyloides stercoralis separated in two clades; one comprising five (5) isolates, that is apparently basal to the rest of the *S. stercoralis* in the evolutionary tree (Figure 6.3). The other two isolates clustered into a clade that included *S. stercoralis* from Australia, Iran, USA and Indonesia.

The evolutionary relationship of *Trichostongylus colubrifomis* shows that the isolate from Kenyan baboon was distinct from those identified from humans and sheep (Figure 6.4). *Oesophagostomum bifurcum* clustered together with other species that were isolated elsewhere from gorilla, humans and other species of monkeys (Figure 6.4). *Trichostrongylus colubriformis* clustered with others that were isolated elsewhere from sheep and humans but showed some separation from the two (Figure 6.4).

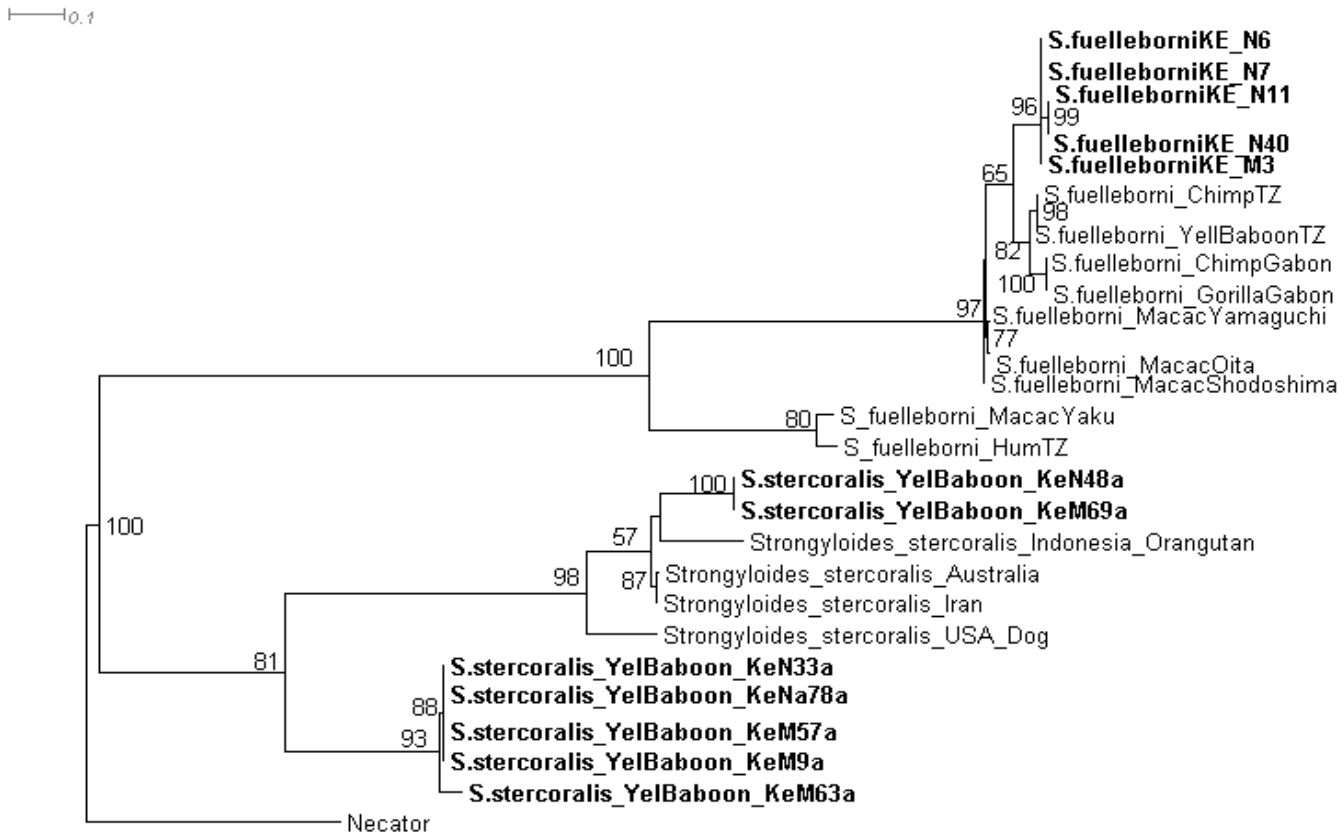


Figure 6.3: Evolutionary relationships of the present isolates (black bold) with *Strongyloides* spp sequences from the Genebank. The rooted maximum likelihood tree based on mtDNA was derived at 1000 bootstrap replicates using *Necator sp* as outgroup. The numbers next to branches represent bootstrap values where values <50% were collapsed.

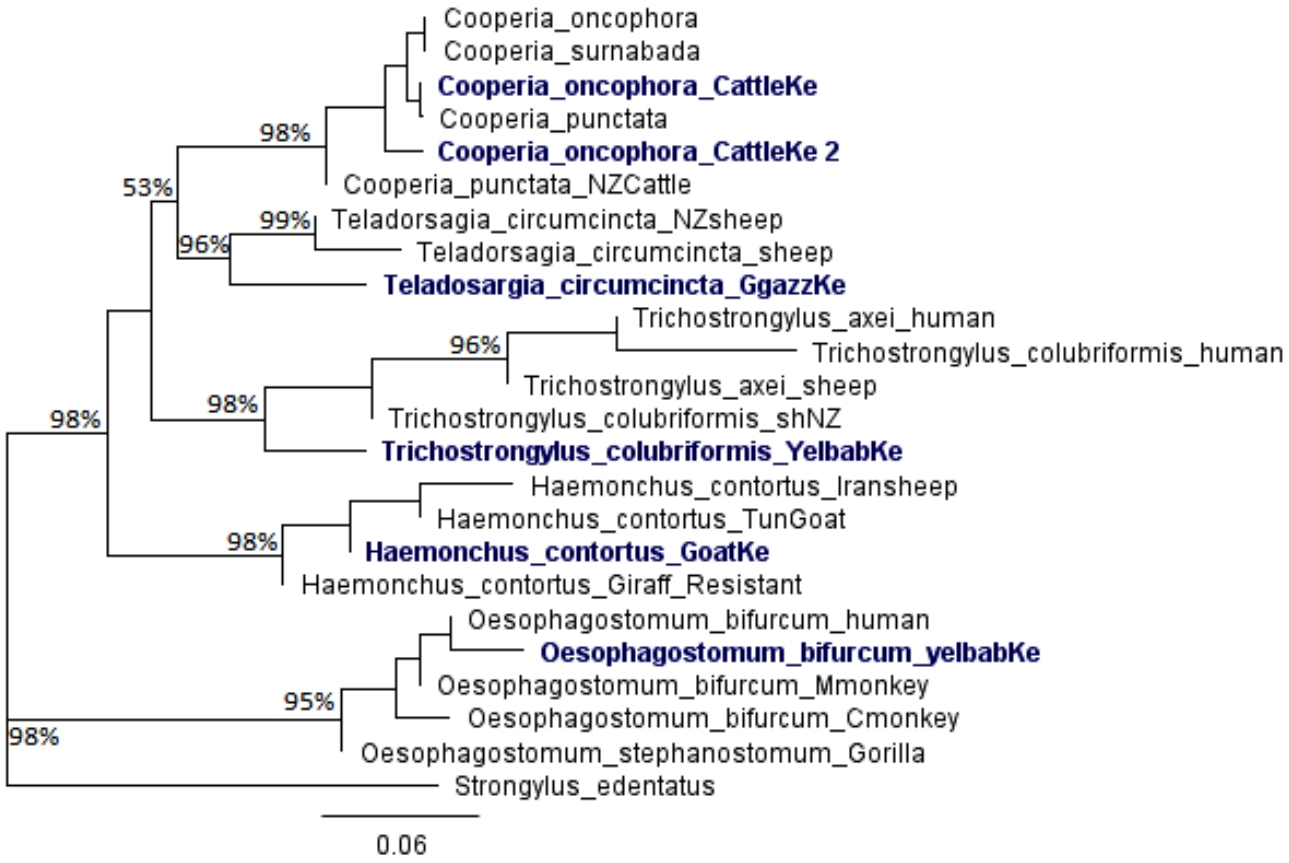


Figure 6.4: Evolutionary relationships of the present isolates (blue bold) with Trichostrongylid sequences selected from the Genebank. The rooted maximum likelihood tree based on ITS of the rDNA was derived at 1000 bootstrap replicates using *Strongylus edentatus* as outgroup. The numbers next to branches represent bootstrap values where values <50% were collapsed.

Cattle and goat were also infected by *Cooperia oncophora* and *Haemonchus contortus*, respectively (Figure 6.4) whereas *Teladosargia circumcincta* was the only nematode identified from Grants' gazelle. *Cooperia oncophora* clustered in a clade that included other isolates of *C. oncophora* and *C. surnabada* as well as *C. punctata* (Figure 6.4). *Haemonchus contortus* showed closer relationship with other species isolated elsewhere from goat sheep and giraffe. *Teladorsargia circumcincta* in Grant's gazelle clustered with other species isolated elsewhere from sheep (Figure 6.4).

6.3.3 Co-occurrence of helminths

The identified Cyathostominae nematosde co-occurred in six out of nine sympatric hosts that include baboon, vervet monkey, impala, Thomson's gazelle, goat and cattle. The other helminths species were found in single hosts. Baboon was the host with the highest helminth species richness as it harboured five different species while the rest of the hosts harboured single helminth species (Figure 6.1; 6.2; 6.3 and 6.4).

6.4 Discussion

6.4.1 Sequence analysis

The two genetic markers, ITSrDNA and MtDNA, were successful in the identification of the larval nematodes. The ITSrDNA gene is increasingly being used to distinguish closely related species of helminths including between strongylid nematodes (Chilton *et al*., 1995; Hung *et al*., 1996; Archie and Ezenwa, 2011; McLean *et al*., 2012). On the other hand, mtDNA gene marker is commonly used for species identification because it undergoes rapid evolution, a trait that enables it to discriminate not only between closely related species but also phylogeographic groups within a single species (Cox and Herbert, 2001; Wares and Cunningham, 2001). However, these genetic markers are rarely used together in a single study or for identification of a single nematode species yet when used in combination they enhance identification output and provide more genetic information (Archie and Ezenwa, 2011; McLean *et al*., 2012). In the present study, only, 6.8% of the total larvae were identified which implies that success rate is very low and it is necessary to begin with high larval numbers. The low number of genotyped larvae was probably a consequence of multiple factors, such as loss of larvae during vaccum evaporation of ethanol and sequencing errors. Therefore, the results from this study do not represent the entire nematode diversity in the Amboseli host community. However, the results were sufficient to address the objectives of this study, which was to determine nematode sharing across hosts.

The most abundant helminth identified in the present study was the *Cyathostominae* nematode, which belonged to the sub-family Cyathostominae (Table 6.1). Members of the sub-family Cyathostominae usually infect a restricted range of hosts that include

elephants, horses, zebra, rhinoceros and donkeys (Anderson *et al*., 2009). However, there are few incidences in which some species in the sub-family Cyathostominae have been recorded outside the expected host range (Hastings *et al*., 1992; Ashford *et al*., 1996). Elephant population in Amboseli, Kenya, harbor diverse species of nematodes in the sub-family Cyathostominae (McLean *et al*., 2012), which could be the source of infection to other sympatric hosts (Table 6.1). Perhaps the few studies on helminth genetics in both wildlife and livestock have contributed to the perception that nematodes in the sub-family Cyathostominae are not found outside their usual host range.

Both species of *Strongyloides* constituted the second most common helminth and were identified from baboon only (Table 6.5). Previous study of helminths in Amboseli baboon found very low prevalence (2%) of unidentified *Strongyloides* spp (Hahn *et al*., 2003), which imply that either of the species of *Strongyloides* have persisted in the Amboseli baboon population. Both species of *Strongyloides* are zoonotic, thus they present public health risk especially to the Maasai herders. Since *S. stercoralis* can penetrate skin to gain human infection coupled by the fact that it can propagate inside humans (endogenous autoinfection), it is one of the most ominous helminths that cause long-lasting suffering (Vadlamudi *et al*., 2006; Prendki *et al*., 2011; Schar *et al*., 2013). Over 100 million people worldwide suffer from *Strongyloides spp.* infections with *S. stercoralis* being the main cause (Anderson *et al*., 2012; Schar *et al*., 2013), though there are human cases of *S. fuelleborni* infections. People who frequently use or share habitats dominated by non-human primates have acquired *S. fuelleborni* thought to be of a non-human primate origin (Hasegawa *et al*., 2010), whereas *S. fuelleborni kellyi* is regarded as a human parasite restricted to Papua New Guinea (Smith *et al*., 1991).

The genus *Oesophagostomum,* which are nodule-causing worms, was the most common natural infection in Kenyan baboons (Vandeberg *et al*., 2009). Previous study of helminths in Amboseli baboon population indicated that they were infected with an unspecified species of *Oesophagostomum* (Hahn *et al*., (2003). This study confirmed that *O. bifurcum* is the species harboured by the Amboseli baboons, which is of particular interest because currently, it a principle zoonotic species (Polderman and Blotkamp, 1995; Ghai *et al*., 2014) beside *O. stephanostomum and O. aculeatum* that incidentally infect people (Blotkamp *et al*., 1993; Polderman and Blotkamp, 1995). Elsewhere, in Ghana, prevalence of *O. bifurcum* has been recorded to be as high as 75-99% in sympatric populations of baboons and Mona monkeys (VanLieshout *et al*., 2005). Perhaps such high prevalence in non-human primates can be associated with human burden for the nodular worm, which is perceived to be localized in West Africa, specifically Togo and Ghana (Polderman *et al*., 1991; Polderman and Blotkamp, 1995). Although, *O. bifurcum* are genetically sub-structured, such that the species infecting baboons cannot infect humans or other host species, recently, a species of *O. bifurcum* that infects both humans and several other non-human primates was identified in Uganda where human-wildlife habitats overlapped (Ghai *et al*., 2014). As such, the presence of *O. bifurcum* in the Amboseli baboon is a potential risk to the pastoralists.

Amboseli baboons were previously recorded to harbor unknown species of *Trichostrongylus* (Hahn *et al*., 2003), however this study confirmed that the baboons were infected with *T. colubriformis*. Although nematodes in the genus *Trichostrongylus* comprise many species of veterinary importance as they are a major cause of ill-health and economic burden in livestock production (Tan *et al*., 2014), some species such as *T.*

colubriformis are considered zoonotic. In areas that human-animal habitats are overlapped, *T. colubriformis* is a perennial public health burden (Boreham *et al*., 1995; Yong *et al*., 2007; Sato *et al*., 2011), which could reflect potential risks that human communities that share habitat resources with baboons in Amboseli ecosystem are exposed to. It is therefore important to determine actual human occurrence of *T. colubriformis* in the Amboseli community.

The genus *Haemonchus* comprises of blood sucking trichostrongylid nematodes, of which *Haemonchus contortus* is the most widespread and known species often associated with huge economic and production losses in the livestock industry, especially in Africa (O'Connor *et al*., 2006; Zajac, 2006; Mekonnen, 2007). The adult worms of *H. contortus* are blood-feeders and drain blood from their ruminant hosts resulting in severe anaemia and even death (Gasser *et al*., 2008). In the present study, the species was identified only from goats. A previous study suggests that *H. contortus* is highly prevalent among pastoralist goats in Kenya (Gatongi *et al*., 1988).

According to Mekonnen, (2007), *Teladosargia circumcincta* is one of the most harmful strongylid nematodes in livestock, particularly sheep and goats in Africa. However, in the present study, the nematode was only found in Grant's gazelle. Presence of *T. circumcincta* in Grant's gazelle is the first record in Kenya but not uncommon elsewhere in other gazelle species or livestock. The genus *Cooperia* comprises Trichostrongyloid nematodes of veterinary importance as they contribute to mixed species helminthosis that leads to production losses in livestock worldwide (Perry and Randolph, 1999; Stromberg *et al*., 2012). In the present study, *C. oncophora* was identified from the pastoralist Maasai cattle and not in other livestock species.

6.4.2 Genetic characteristics of the isolated nematodes

Genetically, the isolated *Cyathostominae* nematodes were monophyletic with genera *Murshidia, Quilonia, Khalilia* (Figure 6.1) and clustered closer to genus *Kiluluma* (Figure 6.2). Limited information is known about *Murshidia, Quilonia, Khalilia* and *Kiluluma*, except their incidence, prevalence, systematics (Lane, 1921; Daubney, 1923; Zumpt, 1964; Boomker *et al*., 1991; Kinsella *et al*., 2004; McLean *et al*., 2012; Beveridge and Jabbar, 2013) and that their host range is restricted among the perrisodactyls and proboscids (Anderson *et al*., 2009). Since all the helminths genera included in the evolutionary trees (Figure 6.1 and 6.2) belong to the sub-family Cyathostominae (Strongylidae), this means that the identified *Cyathostominae* could be a novel genus that is closely related to the proboscid- and rhinocerotid- nematodes in the sub-family Cyathostominae. Further, the identified *Cyathostominae* displayed evolutionary divergence as they separated into three clades (Figure 6.1). This is the first time nematodes of the sub-family Cyathostominae have been isolated from livestock (cattle and goats) as well as in baboons, vervet monkey, impala and Thomson's gazelle.

The evolutionary relationship of *S. fuelleborni* was observed to be strongly influenced by geographical location rather than host taxa (Figure 6.3), an observation that agrees with Hasegawa *et al*., (2010). This means that *S. fuelleborni* populations in Kenya are genetically distinct from those in other countries irrespective of whichever host they are identified from. Yet, interestingly evolutionary rates of *S. fuelleborni* that occur in different parts of Africa were apparently congruent, meaning they are subject to similar drivers of evolution. In contrast, *S. fuelleborni* isolates from populations of Macaque monkeys in Yaku Island suggest they are a distinct lineage from *S. fuelleborni* in Oita,

Yamaguchi and Shodoshima in Japan, an aspect likely to be due to genetic drift (Figure 6.3).

This study also demonstrated the co-occurrence of both *S. stercoralis* and *S. fuelleborni* in a single host species within a particular localized habitat. The co-occurrence is rarely encountered or published, yet such information is of epidemiological importance since both species are zoonotic. It was also interesting to observe that though *S. stercoralis* was identified from a single host population, their evolution signals separate divergence with some having undergone more genetic changes than others (Figure 6.3). Since, 1989, there are only four published surveys on prevalence of *S. stercoralis* in Kenya (Schar *et al*., 2013), which is quite minimal, thus results from this study will greatly enrich the epidemiological information on this helminth in Kenya.

The evolutionary relationship of the *O. bifurcum* in the present study relative to those from other host species (Figure 6.4) agrees with the theory of genetic sub-structuring which have been supported phenotypically and genetically (de Gruijter *et al*., 2004; 2005). Therefore, the *O. bifurcum* identified from Amboseli baboon were distinct. Sub-structuring means that populations of *O.bifurcum* in baboon, Mona monkey, Cynomolgous monkey and humans, differ in both salient morphological features (de Gruijter *et al*., 2002) and genetic sequences (de Gruijter *et al*., 2004; 2005). Nevertheless, recent detection of multiple cryptic *Oesophagostomum* species that could co-infect humans and other non-human primates (Ghai *et al*., 2014) calls for advanced genetic studies on these helminths.

The *Trichostrongylus colubriformis* that was identified in baboon was distinct from the rest of the *Trichostrongylus* spp. including those identified from humans (Figure 6.4). The *H. contortus* from goat clustered with other species identified elsewhere but was closer to those from goats rather than from sheep or giraffe, which may signify host clustering (Figure 6.4). The species of *T. circumcincta* from Grant's gazelle were apparently distinct and more basal from those of identified elsewhere from sheep (Figure 6.4). This may imply that the species identified in the gazelles have not undergone much genetic alterations as compared to those in sheep. The two *C. oncophora* isotypes identified from Maasai cattle suggests within-population variation of *C. oncophora* of which one is distinct while the other is closer to *C. punctata* (Figure 6.4).

6.4.3 Helminth overlap

Results from this study demonstrate that hosts living in sympatry share some species of nematodes or rather some nematodes species inhabit multiple hosts. Cross transmission or sharing of nematodes between host species is not uncommon especially between closely related hosts e.g. bovids, (Ezenwa, 2003; Ocaido *et al*., 2004; Archie and Ezenwa, 2011) but rare in hosts of distant ancestry. Common ancestry facilitates parasite sharing between related hosts because of similarity in their mechanisms of immune defense as well as life-history traits (Page, 2003). For a parasite to jump host species, there must be constant exposure to the new host, it must undergo process of adaptations and colonization to evade host immunity and gain physiological needs from the new host (Turner and Elena, 2000; Pedersen and Davies, 2010). Therefore, the co-occurrence of the identified Cyathostominae nematodes in both non-human primates and ungulates (Figure 6.1 and 6.2), hosts that are distantly related and also novel in terms of the usual

host range for nematodes of the sub-family Cyathostominae, demonstrates host shift and consequently host range expansion (Antonovics *et al*., 2002). Habitat overlap is therefore critical for nurturing events for host shift, a phenomenon that drives emergence of new infectious diseases (Jones *et al*., 2008; Pedersen and Davies, 2010).

In summary, results from this study reveal that particular helminth species may dominantly use multiple animals as hosts. The *Cyathostominae* nematodes suggests it could be a novel helminth species. Baboons in Amboseli harbor several species of helminths regarded as zoonotic, hence of public health concern. This study has confirmed co-occurrence of both *S. fuelleborni* and *S. stercoralis* in Kenyan baboon. Further, evolutionary relationships could be used to resolve taxonomical ambiguities of some helminth species.

CHAPTER SEVEN: CHARACTERISTICS OF THE CYATHOSTOMINAE NEMATODES

7.1: Introduction

This section is an expansion of chapter 6, which is to specifically discuss further the identified nematode in the sub-family Cyathostominae. This section provides more information about the phenotypic traits and evolutionary relationships of the Cyathostominae.

7.2: Phenotypic characteristics

The *Cyathostominae* nematode was extracted from faecal culture and examined under the microscope as described in Chapter 6. Both larval and sexually-mature life stages were observed meaning that the development cycle of the nematode was rapid whereby the 10-12 day culture had both life stages. The sexually mature stages were gravid females that harbored both ova and larvae (3-4) *in uteri* (Figure 7.1), meaning that the nematode had an ovoviparous reproductive trait.

While examining the worms under the microscope, some laid live larvae after undergoing strong somatic contraction which was followed by death of the female. Morphometrics of this worm were recorded. The largest diameter was the section contiguous to vaginal opening, which measured 77.57 µm (Figure 7.1A and E). The oesophagus had four sections with two bulbs spanning the length from oral cavity to end of posterior bulb (Figure 7.1C; Table 1). Tail length measured from the position of anus to the tip of the tail was 104.91µm. Intra-uterine larvae were in constant wriggle, stretched out along the uterine cavity and aligned parallel to each other. The eggs *in uteri* were irregularly ellipsoidal, probably depicting different development stages. Salient features of this Cyathostominae worm were compared with features of other *Rhabditis hominis* and *Strongyloides stercoralis*, which closely resemble it (Table. 7.1).

Figure 7.1: Microimage of the Cyathostominae nematode: (A) Middle section of the worm showing immature eggs and larva in the uterus, 400x (B) whole worm, 40x (C) Anterior part of the worm showing the two bulbs of the oesophagus, 400x (D) Posterior part of the worm showing the tail, 400x (E) whole worm, 400x. Scale: 50µm.

Table 7.1: Differential comparison of morphology of the viviparous nematode isolated in the present study, *Rhabditis hominis* and free-living *Strongyloides stercoralis*.

Present study	Refs: Kobayashi, 1920; Sandground, 1925	Refs:Sandground, 1925 and Hasegawa *et al*., 2010
Isolated larva	*Rhabditis hominis*	*Strongyloides stercoralis*
Female **Length:** 782.56-794.41µm **Width**: 77.57µm **Buccal cavity**: not measured **Tail:** 104.91-116.32µm long **Reproduction:** Ovo-viviparous **Eggs:**28.1-48.68µm by 15.23-29.35µm often arranged in single row in each uterus; 6-8 in number	Female **Length:** 1400-2000µm **Width:** 120µm **Buccal cavity**: 20µm long **Tail:** 170-224µm long **Reproduction:** Ovo-viviparous **Eggs**: 24-44µm by 28-32µm often arranged in double row in each uterus; 20-50 in number	Female **Length:** 1000-1200µm **Width:** 50µm **Buccal cavity**: 13µm long **Tail:** 125-155µm long **Reproduction**: Oviparous **Eggs:** 42-46µm by 33-36µm often arranged in single row in each uterus; 16-18 in number
Female oesophagus: 132.72-144.62µm long Anterior canal: 66.19µm long Anterior bulbus: 18.76µm wide Posterior canal: 64.08µm long Posterior bulbus: 20.97µm wide	**Female oesophagus:** 170-200 µm long Anterior canal Anterior bulbus Posterior canal Posterior bulbus: 20µm wide	**Female oesophagus**: 111-146µm long

Larva (young rhabditiform) **Length**: 253.84µm **Width:** 14.84µm **Buccal cavity:** not measured Larva developed to sexual phase	Larva (young rhabditiform) **Length:** 240-300µm **Width:** 120-30µm **Buccal cavity:** 15-19µm long Larva always develops to rhabditiform sexual adult	Larva (young rhabditiform) **Length:** 200-250µm Width: 16µm Buccal cavity: 8-10µm long Larva always develops either into sexual intermediate rhabditiform generation or metamorphoses directly into the filarial larva

7.2: Genetic characteristics

The high bootstrap values for the phylogenetic relationship between the larval Cyathostominae and other known genera of the sub-family Cyathostominae obtained from Genebank indicated they belong to the sub-family (Figure 6.1 and 6.2). Based on mtDNA, the larval Cyathostominae clustered with *Murshidia, Quilonia* and *Khalilia* and displayed recent genetic changes that are far removed from equine nematodes (Figure 6.1). However, based on ITS gene, the position of the larval Cyathostominae on the tree alternated and clustered separately away from *Murshidia, Quilonia* and *Khalilia* but closer to the clades of *Cylicocyclus* and *Kiluluma* (figure Figure 6.2).

7.3: Discussion

7.3.1: Host range

The usual hosts for nematodes in the sub-family Cyathostominae belong to two broad categories of hosts: Perrisodactyls that include rhinoceros, zebra, donkey, horse, warthog and tapir and the Proboscidae that comprises both African and Asian species of elephants (Lane, 1921; Zumpt, 1964; Boomker *et al*., 1991; Kinsella *et al*., 2004; Anderson *et al*., 2009; McLean *et al*., 2012). Within the Perrisodactlys, nematodes that infect equines, which includes horse, zebra and donkey, are referred to as cyathostominae and includes over 50 species of which some were included in the phylogenetic tree in the present study (Figure 6.1 and 6.2). Elephants and rhinoceros are the main hosts that share genera *Murshidia, Khalilia, Quilonia* and *Kiluluma* (Lane, 1921; Zumpt, 1964; Kinsella *et al*., 2004; McLean *et al*., 2012). However, few incidences of *Murshidia pugnicaudata and M. hamata* have been identified in warthogs (Daubney, 1923; Boomker *et al*., 1991) while *Murshidia devians* have been identified in both lowland (*Gorilla gorilla*) and mountain (*Gorilla beringei*) gorillas (Campana-Rouget, 1959; Hastings *et al*., 1992; Ashford *et al*., 1996). Other Cyathostominae genera like *Sauricola* and *Chapinniela* are also known to have constricted host range in the American tortoises, *Testudo denticulata* and *Gopherus polyphemus* (Lichtenfels and Stewart, 1981). The only species ever recorded in an antelope is *Eucyathostomum webbi* in white-tailed deer (Pursglove, 1976).

This background suggests that although nematodes in the sub-family of Cyathostominae have a narrow host-range, the few incidences that they have occurred outside their traditional host range mean that they could adapt to colonize new hosts. Results from this

study demonstrate for the first time occurrence of Cyathostominae in livestock (cattle and goats) as well as in baboons, vervet monkey, impala and Thomson's gazelle.

7.3.2: Phenotypic characteristics

Basic phenotypic traits, which included presence of rhabditiform larvae, were similar to those of *Rhabditis hominis* and the free-living generation of *Strongyloides stercoralis* (Table 7.1). However, the identified nematode differs from *S. stercoralis* because of its ovoviviparity. On the other hand, *Rhabditis hominis* is ovoviviparous (Kobayashi, 1920; Sandground, 1925) meaning that the identified Cyathostominae and *Rh. hominis* have similar reproductive traits. Remarkable deviation from the identified Cyathostominae is that most of the species of *Rhabditis* are free-living while the former were identified from six host taxa. Previously, the association of *Rhabditis* spp. with vertebrates including humans were disputed and claimed to be spurious infections or fecal contaminations by coprophagous flies (Kobayashi, 1920; Sandground, 1925), however cases of infections in livestock (Msolla *et al*., 1993; Duarte *et al.* 2001) and increasing cases of human infections (Goldsmid, 1967; Meamar *et al*., 2007; Anderson *et al*., 2009) suggest otherwise, that it can be parasitic. Recently, the first human case of outer ear canal infection by *Rhabditis* spp. was published (Teschner *et al.* 2014). Overall, the identified Cyathostominae was distinct from *S. stercoralis* and *Rh. hominis.*

The ovoviviparity, which was displayed by the Cyathostominae nematode, was a rare reproductive trait that according to Chen and Caswell-Chen, (2004) is referred to as bagging or facultative vivipary. This is a facultative feature in which hatching of ova into larvae occurs intra-uterine and larvae are laid out (vivipary) followed by death of the

female (matrotrophy). Matrotrophy occurs due to severe internal damage as developing larvae ingests maternal tissues (Chen and Caswell-Chen, 2004).

This reproductive option is seen in *Caenorhabditis elegans* and is thought to be a survival strategy induced during nutritional stress or adverse environmental conditions (Chen and Caswell-Chen, 2004). Vivipary has been observed even among the oviparous parasitic nematodes such as the well-known *Haemonchus contortus* (Ayalew and Murphy, 1986) and commonly in rhabditid nematodes (Kobayashi, 1920; Sandground, 1925; Sudhaus, 1974; Belogurov *et al*., 1977; Kampfe *et al*., 1993), which points out the facultative potential for viviparity among nematodes (Blackburn, 1998; Chen and Caswell-Chen, 2004).

7.3.3: Genetic characteristics

The isolated larval Cyathostominae fitted very well in the evolutionary relationship of the sub-family Cyathostominae, which advances understanding of the relationship among nematodes in the sub-family. For instance, the equine cyathostominea, are the most studied group of the Cyathostominae and yet their evolutionary relationships is still equivocal and their genetics are incongruent with morphological traits (Lichtenfels, 1979; Dvojnos, 1982; Love *et al*., 1992; McDonnell *et al*., 2000; Hung *et al*., 2000). Specifically, the relationships between the 'large' and 'small' equine *Strongylus* were not explicit and conclusive. However, results in this study show that *Strongylus vulgaris* and *S. equinus* (Strongylinae), which are 'large strongyles' and here used as 'out-groups', are truly ancestral to other genera of Cyathostominae (Figure 6.1 and 6.2), and consistent with the traditional phenotypic classifications (Lichtenfels, 1980; 1998).

In the present study, it was also noted that the ITS genes of the genera *Kiluluma* that is restricted to rhinoceros and *Cylicocyclus* that infects equines show similarity in their evolutionary patterns (Figure 6.2). Further, the equine cyathostominea were paraphyletic (Figure 6.1 and 6.2) which contradicts previous notion that the over 50 species of cyathostominea (Lichtenfels *et al*., 1998) are monophyletic (Dvojnos, 1982). This is supported by the fact that the genus *Cylicocyclus* consistently separated from other equine genera such as *Cyathostomum*, *Coronocyclus* and *Cylicostephanus* (Figure 6.1 and 6.2), a pattern that is consistent with previous studies (McDonnell *et al*., 2000; Hung *et al*., 2000). Moreover, evolutionary relationship of *Triodontophorus serratus* has always been problematic with suggestions that it belongs to the family Strongylinae (large equine strongyles), but in the present study it clustered with the family Cyathostominea (small equine strongyles), an observation that agreed with some previous studies (Hung *et al*., 2000; McDonnell *et al*., 2000). The problem with classification of *T. serratus* is that its phenotypic traits are incongruence with phylogenetic lineage. However, Hung *et al*., (2000) postulated that *T. serratus* should be classified as Cyathostominea, which the phylogenetic analysis of this study is in agreement with. The position of *Tridentoinfundibulum gobi* in the present study, also concurs with previous studies (McDonnell *et al*., 2000) and though its biology is not well understood, it is apparent that it belongs to the Cyathostominea.

In summary, there are several genera whose taxonomy is still equivocal probably due to the fact that separation of equine nematode families Strongylinae and Cyathostominea (Cyathostominae) were based on arbitrary phenotypic features (Lichtenfels *et al*., 1998) which is incongruent with the emerging phylogenetic information (Hung *et al*., 2000;

McDonnell *et al*., 2000). According to Swofford, (1991) there are numerous examples of incongruence between phylogenies based on molecular and morphological data sets. This means that even though vivipary has not been seen among Cyathostominae or some of the phenotypic traits of the isolated Cyathostominae nematode, its evolutionary relationship suggests it is a member of the sub-family Cyathostominae. Perhaps the different biological features of the isolated Cyathostominae from the rest of the sub-family could be adaptive traits for survival in multiple hosts.

CHAPTER EIGHT: GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

8.1. General discussion

The overall objective of this study was to determine the effects of habitat overlap between sympatric hosts on transmission of helminths in Amboseli ecosystem, Kenya. The first specific objective was to determine the degree of habitat overlap among the social groups of baboons and between baboons and the rest of the sympatric host species. The results suggested that both the size of baboon habitats and degree of habitat overlap across social groups were heterogenous (Table 3.1 - 3.4). In the dry season, the baboons in Amboseli occupied smaller habitat ranges characterized by high degree of overlap among the social groups (Table 3.1 and 3.2) whereas in the wet season, the habitat ranges were expanded with less degree of overlap (Table 3.3 and 3.4). This seasonally-driven vacillation in the size of habitat range in the Amboseli baboon is a common pattern among the grassland dwelling savannah baboon populations and less in forest dwellers (Norton *et al*., 1987; Wahungu, 1998; 2001). The pattern demonstrates the elastic trait of habitat range size, most likely subject to climatic conditions (Pearce *et al*., 2013).

The degree of habitat overlap between the social groups (Table 3.1-3.4) and between baboon and alternatives hosts (Table 3.5 - 3.8) was relatively lower at the core habitat range (50% MCP) compared to overlap at the maximum habitat range (100%MCP). The area of habitat that territorial animals use for most of their daily activities represents their core habitat and this area is usually defended aggressively from other intruding social groups including conspecifics (Cowlishaw, 1992; Mertl-Millhollen, 2006; Crofoot and

Wrangham, 2009). However, factors or strategies that reduce the degree of habitat overlap between hosts species are not clearly understood (Kukielka *et al*., 2013).

The degree of habitat overlap between baboon and other alternative hosts was also heterogenous suggesting that some social groups experienced greater interaction or disturbance with heterospecifics compared to other social groups. Although, season influenced the degree of overlap between baboon social groups, degree of overlap between baboon and heterospecifics lacked a clear seasonal pattern (Table 3.5-3.8), which could be due to spatial and temporal niche partitioning whereby different host species use space and resources differently at different times (Albrecht and Gotteli, 2001).

The second objective was to determine helminth prevalence, abundance and species richness in all the sympatric hosts. In this study, both sedimentation and floatation methods were used to determine helminths prevalence, abundance and species richness. Most studies report of helminth prevalence based on sedimentation method only, however, in this study, prevalence was determined by both sedimentation and floatation methods. Results showed that prevalence of helminths across host species were significantly different by both sedimentation (χ^2 = 200.37, df = 8, p = 0.0001) and floatation (χ^2 = 54.505, df = 8, p<0.0001) methods. This means that although multiple hosts may co-occur in the same habitat, the rates of helminth infection are distinct even among closely related hosts. This may be attributed to differences in host susceptibility and exposure to infective stages of the helminths, due to the hosts' feeding habits. It was also noted that prevalence of helminths across hosts differed significantly during dry and

wet season (χ^2 = 23.87, df = 1, p = 0.0001), suggesting that seasonality had universal influence on the transmission of helminths across host species.

Prevalence of helminths across the baboon social groups were significantly different by both sedimentation (χ^2 = 22.43, df = 5, p = 0.0001) and floatation (χ^2 = 27.754, df = 5, p<0.0001) methods. This finding suggests that a social unit is an important factor that influences transmission of helminths and that each social unit suffers different rates of infection. Multi-level societies, such as those of baboons, social groups are important structures of the population and are usually heterogenous in their size and density, age and sex ratio as well as inter-individual contacts, factors that drive parasitism or disease epidemiology (Caillaud *et al*., 2013).

It was noted that when floatation method was used, there was no significant difference (χ^2 = 1.680, df = 1, p = 0.195) in prevalence of helminths in the dry or wet season, which implies that seasonality did not have effect on helminths transmission in baboons. In contrast, prevalence of helminths determined by sedimentation method showed an explicit seasonal pattern whereby dry season was marked by higher prevalence (χ^2 = 13.56, df = 1, p = 0.019) compared to wet season (χ^2 = 18.26, df = 1, p = 0.003). Seasonality usually elicits strong effects on transmission rates of helminths, which suggests that results by sedimentation method reflected the expected pattern (Thomas *et al*., 2002)

Prevalence of helminths that were determined by floatation method across host species were significantly higher (χ^2 = 157.472, df = 1, p<0.001) than when determined by sedimentation method. This means that interpretation and comparison of results on

prevalence of helminths with previous published studies should consider the parasitological method used to avoid bias. The floatation by centrifugation method that was used in this study is most likely more sensitive to detect helminth ova compared to sedimentation method. Nevertheless, sedimentation method was superior in detecting more richness of helminth species (χ^2 = 132.703, df = 5, p<0.001) than floatation method. According to Cohens' kappa statistic, the concordance between floatation and sedimentation methods was low (0.101), meaning that each test method was distinct. However, since each method had advantage over the other, it is recommendable to use both methods, particulalrly sedimentation method for species richness and floatation technique for prevalence.

The results indicated that abundance of helminths varied across host taxa and across social groups of baboon. This means that some host taxa or baboon social groups harboured relatively low abundance of helminths compared to others. Arneberg *et al*., (1998) explains that such intra-species and inter-species variation in abundance of helminths is a function of host population density whereby helminth abundance is positively associated with host density. In the present study, there was lack of association between abundance of helminths and density of each social group measured at 100% MCP (r_s = 0.600, n = 6, p = 0.242) and at 50% MCP (r_s = 0.771, n = 6, p = 0.103) levels of core habitat. The association, though statistically insignificant, showed a positive trend in which high density was predictive of high abundance of helminths (Figures 5.3 and 5.4).

Helminth species richness across the host species community ranged from two to eight, with mean of 5.1 ± 1.9. The Amboseli host community harboured a rich diversity of

helminths that included nematodes, trematodes and cestodes. Nematodes were the most common taxa of helminths in the Amboseli host community. Specifically, strongylids was the most common group of nematodes that co-occurred across the nine host species.

The third objective was to determine the influence of habitat overlap among baboon groups and between baboons and alternative host species on helminths infection rates. Degree of habitat overlap between home ranges was used as an index of degree of habitat overlap between baboon social groups. Since degree of overlaps between home ranges of baboon groups were not variable at 100% MCP, only the degree of habitat overlaps at 50% MCP were tested for association with helminth prevalence, abundance and species richness. The results indicated a lack of statistical association between degree of habitat overlap across baboon social groups and their helminth prevalence, abundance and species richness ($p > 0.05$). This finding suggests that social groups that received more immigrants did not suffer more infection than those that received fewer immigrants. A similar observation was recorded among the Howlers monkeys (Gonzalez-Hernandez *et al*., 2011), which implies the pattern is common among the socially structured societies of non-human primates. Since home ranges of baboons tend to be elastic (Pearce *et al*., 2013) whereby they exhibit spatio-temporal expansion and constriction, intrusion or immigration by members of other social groups is short lived and may not influence density-dependent factors that determine helminth transmission.

In addition, the degree of habitat overlap between baboon and alternative hosts was based on dung pile count and frequency of sighting alternative hosts in baboon home ranges. The association between dung pile counts, mean frequency of animal sightings, Shannon-Wiener diversity index, host species diversity and both helminth prevalence and

abundance in baboons were tested. Statistical analysis showed that at 100% or 50% MCP of baboon home range, the degree of habitat overlap (based on dung pile counts and frequency sightings) between baboon and alternative hosts did not significantly influence both helminth prevalence and abundance. These results suggest that the degree of habitat overlap within species and across host taxa do not influence helminth prevalence and abundance. Increased degree of habitat overlap, which can be a surrogate for increased density, is supposed to positively correlate with prevalence and abundance. Thus results from this study seems to contradict the epidemiological theory of the positive association between host density and both prevalence and abundance (Arneberg *et al*., 1998).

Degree of habitat overlap (based on host diversity and Shannon-Wiener diversity index) was negatively associated with helminths species richness in baboons, meaning that baboons whose home range were occupied by more diverse host species harboured less helminth species richness. The association between host diversity and helminths richness is not clear but displays a range of interactions that include lack of association (Watve and Sukumar, 1995; Ezenwa, 2003), negative association (Keesing *et al*., 2006; Ostfeld and Keesing, 2012) and a positive association (Hechinger and Lafferty, 2005). Such variable interactions suggest that there are other stronger factors driving the relationship between host diversity and helminth species richness.

The fourth objective was to genetically determine the species of nematodes that are shared among sympatric baboons, vervet monkeys and ungulates in Amboseli ecosystem. The results revealed co-occurrence of multiple species of nematodes that varied in their infection rates across hosts. It was interesting to note that in the host community, there was a dominant helminth that co-occurred in many hosts. Parasitological analysis

identified strongylids as the most co-shared helminth in the host community, however, besides, *Trichuris* spp (Table 4.11) genetic analysis revealed that Cyathostominae were indeed the most shared single nematode species.

The nematode in the sub-family Cyathostominae could be a novel species since its genetic relationship was inclined towards Cyathostominae but morphological traits were incongruent to the the traits of the sub-family. Morphological and genetic incongruency is not a rare feature in organisms especially with the advancement of genetic tools that expose evolutionary relatedness (Swofford, 1991). It is worth noting that some species within the sub-family Cyathostominae, such as *Triodontophorus serratus* and *Tridentoinfundibulum gobi*, still have vague taxonomy due to incongruency between morphological and genetic traits (McDonnell *et al*., 2000).

8.2 Conclusions

Objective 1: This study determined that the degree of habitat overlap between baboon social groups was variable, with more overlaps in the dry season compared to the wet season. This is the first record of degree of habitat overlap in a baboon population.

Objective 2: The host community was infected with a rich diversity of helminths, whose prevalence and abundance was variable between species and within species as well as between seasons. Interestingly, the seasonal pattern in prevalence was heterogenous. Sedimentation method of faecal assessment yielded higher helminths species richness compared to floatation method, whereas the latter yielded higher helminth prevalence than the former method.

Objective 3: There was no significant association between degree of habitat overlap with helminths prevalence and abundance. Specifically, Baboon social groups with more overlapping alternative hosts did not have more or less helminths prevalence or abundance than those with fewer alternative hosts. In contrast, increased habitat overlap had a negative association with helminths species richness, meaning that social groups with more diverse hosts harboured relatively lower species richness

Objective 4: Multiple species of nematodes were identified however, Cyathostominae was dominantly shared across hosts, of which some are new hosts for nematodes in the sub-family Cyathaostominae. This implies that in a host community, there is a dominant nematode that cross-infects multiple hosts.

Overall, this study identified the following for the first time in Kenya: *Primasubulura* sp., *Teladosargia circumcincta* and a Cyathostominae. The nematode, Cyathostominae is

putatively novel, but further, its occurrence in new host range imply host shift. This study also identified both *strongyloides stercoralis* and *S. fuelleborni* in a single baboon population. This is the first study to determine helminths infection at the wildlife-livestock interface where ungulates and non-human primates and helminths are shared within and across host phylogenies.

8.3 Recommendations

- The biology, genus and species of the viviparous Cyathostominae identified in this study need further investigation.
- It is important to use both coproscopic parasitological methods and genetic techniques to study helminths epidemiology at the wildlife-livestock interface.
- Zoonotic importance of some of the nematodes, such as *Trichostrongylus colubrifomis, Oesophagostomum bifurcum, Strongyloides stercoralis* and *S. fuelleborni* needs to be investigated further.

9.0. REFERENCES

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APPENDICES

Appendix 1: Alignment image by *Jalview* software of the viviparous cyathostominae nematode and Genebank sequences of other species in the sub-family Cyathostominae.