ZOONOTIC GASTROINTESTINAL HELMINTHS AND HEMOPARASITES OF BABOONS IN TANA RIVER, TSAVO AND LAIKIPIA, KENYA

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FEBRUARY 2017
DECLARATION

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

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DEDICATION

This thesis is dedicated to my family for patience and support and those dedicated to research to improve human health.
ACKNOWLEDGEMENTS

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# ABBREVIATIONS AND ACRONYMS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>ANOVA</td>
<td>Analysis of Variance</td>
</tr>
<tr>
<td>BFV</td>
<td>Bovine Foamy Virus</td>
</tr>
<tr>
<td>CDC</td>
<td>Center for Disease Control</td>
</tr>
<tr>
<td>DCs</td>
<td>Dendritic cells</td>
</tr>
<tr>
<td>df</td>
<td>Degrees of freedom</td>
</tr>
<tr>
<td>EDTA</td>
<td>Ethylene deamine tetra acetic acid</td>
</tr>
<tr>
<td>EFV</td>
<td>Equine Foamy Virus</td>
</tr>
<tr>
<td>FFV</td>
<td>Feline Foamy Virus</td>
</tr>
<tr>
<td>FV</td>
<td>Foamy virus</td>
</tr>
<tr>
<td>HB</td>
<td>Hemoglobin concentration</td>
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<tr>
<td>HIV</td>
<td>Human Immunodeficiency Virus</td>
</tr>
<tr>
<td>IBD</td>
<td>Inflammatory Bowel Disease</td>
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<tr>
<td>IL</td>
<td>Interleukin</td>
</tr>
<tr>
<td>IPR</td>
<td>Institute of Primate Research</td>
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<tr>
<td>KEMRI</td>
<td>Kenya Medical Research Institute</td>
</tr>
<tr>
<td>KWS</td>
<td>Kenya Wildlife Service</td>
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<tr>
<td>LF</td>
<td>Lymphatic Filariasis</td>
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<tr>
<td>Ltd</td>
<td>Limited</td>
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<tr>
<td>MCH</td>
<td>Mean Corpuscular Hemoglobin</td>
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<tr>
<td>MCHC</td>
<td>Mean Corpuscular Hemoglobin Concentration</td>
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<tr>
<td>Abbreviation</td>
<td>Full Form</td>
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<td>--------------</td>
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<tr>
<td>MCV</td>
<td>Mean Corpuscular Volume</td>
</tr>
<tr>
<td>NACOSTI</td>
<td>National Council for Science, Technology and Innovation</td>
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<tr>
<td>NHP</td>
<td>Non – human primates</td>
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<tr>
<td>NTDs</td>
<td>Neglected Tropical Diseases</td>
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<tr>
<td>PAN</td>
<td><em>Papio anubis</em></td>
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<tr>
<td>PCR</td>
<td>Polymerase Chain Reaction</td>
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<tr>
<td>PCT</td>
<td>Preventive Chemotherapy</td>
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<tr>
<td>PCV</td>
<td>Packed Cell Volume</td>
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<tr>
<td>RBC</td>
<td>Red Blood Cell</td>
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<tr>
<td>SFV</td>
<td>Simian Foamy virus</td>
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<tr>
<td>SIV</td>
<td>Simian Immunodeficiency Virus</td>
</tr>
<tr>
<td>STH</td>
<td>Soil Transmitted Helminthes</td>
</tr>
<tr>
<td>Treg</td>
<td>T regulatory lymphocytes</td>
</tr>
<tr>
<td>TRPNR</td>
<td>Tana River Primate National Reserve</td>
</tr>
<tr>
<td>TWNP</td>
<td>Tsavo west</td>
</tr>
<tr>
<td>US</td>
<td>United States</td>
</tr>
<tr>
<td>USD</td>
<td>United States dollar</td>
</tr>
<tr>
<td>USDA</td>
<td>United states Department of Agriculture</td>
</tr>
<tr>
<td>WBC</td>
<td>White Blood Cell</td>
</tr>
<tr>
<td>WII</td>
<td>Wildlife Institute of India</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health organization</td>
</tr>
<tr>
<td>$\chi^2$</td>
<td>Chi square</td>
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Zoonotic pathogens are among the most important causes of ill health in humans all over the world. In Kenya, the encroachment of wildlife habitats has led to increased interaction between humans and non-human primates especially baboons hence potential for zoonoses transmission. However, a risk analysis for these zoonoses had not been undertaken in Kenya. The current study aimed to investigate hemoparasites and gastrointestinal parasites of olive baboons (Papio anubis) at the human–baboon interface in Tsavo West National Park, Tana River Primate Reserve and Mutara Ranch in Laikipia County. Laikipia baboons were used to study hematological responses to helminth treatment and pathology. Questionnaire survey was conducted in Tana River and Tsavo on risk factors associated with zoonoses. Baboons were trapped in the wild, sampled for blood, feecal, ectoparasites and thoroughly examined physically. Blood smears were prepared and examined for hematology and haemoparasites. Uncoagulated blood was used for confirmation of haemoparasites with Polymerase Chain Reaction (PCR). Feecal samples were screened for helminth oocysts. Helminths detected included Oesophagostomum, Strongyloides, Trichuris and Enterobius with the highest frequencies being 80.5%, 77.8%, 27.5% and 14.8% respectively in the three study sites. Schistosoma mansoni was only detected in Tsavo baboons (2.1%). There was a significant difference in prevalence of the helminths among the three sites (P<0.05) with Laikipia having the highest frequencies followed by Tana then Tsavo. Infection intensities were light in all the study sites. Following treatment, all the leucocyte parameters did not change significantly (P<0.05) but there was a significant increase in erythrocyte indices (P<0.05) except MCV (P>0.05) though all were still within normal ranges. Hemoparasites were detected by PCR in Tsavo and Tana River baboons only and these included Hepatocystis kochi (90% and 87%), Babesia (10.8% and 16.7 %), and Entopolypoides (8.7% and 5%) respectively. There was an association between H. kochi infection and lymph node enlargement as well as fever ($\chi^2$>3.84, df=1, P<0.05) in both Tana River and Tsavo baboons. Tissue pathology due to helminth revealed nodular lesions with epithelial necrosis due to Oesophagostomum in large intestines, lung fibrosis due to Strongyloides infection and intussusception attributed to Trichuris infection. Schistosoma mansoni infection revealed granuloma formation, parenchymal and periportal fibrosis in liver. Risk factors of zoonosis in Tsavo and Tana River included sharing of river water (6%, 76%), baboon crop raids (65% and 75.1%) and baboon livestock predation (92.9% and 91.8%), consumption of left overs from crop raids/ predation (66%, 19.8%), lack of knowledge of zoonoses (81% and 92%), monkey meat consumption (12% and 2%) respectively. Significant factors included baboon crop raids, left over consumption, and lack of knowledge on zoonosis (P<0.05) in both sites. There was a high interaction between humans and baboons which increased the risk of zoonoses transmission. Public education on zoonosis, training of health staff on zoonoses and improving compensation due to losses caused by baboons is highly recommended to reduce human-wildlife the interaction and potential for zoonoses transmission.
CHAPTER ONE: INTRODUCTION

1.1 Background information

The rapid increase in human population and the unprecedented mobility of wildlife has dramatically increased the number of people interacting with non-human primates (NHPs). Human migration and deforestation are rapidly depleting the remaining natural habitats of wild NHPs, squeezing the animals into localized areas (Wallis and Lee, 1999; Gillespie et al., 2005). The situation is compounded by the ever increasing bush-meat trade where NHPs are trapped and butchered by several communities in Africa. Morphological, physiological, genetic and behavioral similarities between humans and NHPs have made possible the bi-directional transmission of a variety of infectious agents between humans, NHPs and livestock (Wallis and Lee, 1999). Baboons are highly destructive and have high interaction with humans (Butynski and Mwangi, 1994); this makes them more prone to bush meat trade and also as agents of zoonoses transmission.

Pathogens that are relatively non-virulent in NHPs, for example HIV in chimpanzees, have been introduced into human beings where they have caused devastating effects (Gao et al., 1999). On the other hand, endemic human diseases such as influenza, tuberculosis, chicken pox, measles and scabies can cause mortality rates greater than 90% in captive NHPs (Mansfield and King, 1998). In spite of possible emergence of zoonoses transmission between NHPs and humans, the frequency of transmission of such infections in wild primates with human contact remains un-quantified and
organized efforts to prevent disease transmission are lacking (Kortlandt, 1996). Nearly all of the emerging infectious threats are zoonoses which mainly originate from wildlife with NHPs being the main sources (Murphy, 2008). Studies have implicated hunting, eating and preparation of primate bush-meat in central and western Africa in the transmission of simian foamy virus and Ebola virus (Hussain et al., 2003; Wolfe et al., 2005). In Tanzania, people living near Gombe National Park have been infected with polio, an enterovirus, transmitted through water contamination by feaces suspected to be from Non-human primates (Travis et al., 2006). Non-human primates in close contact with humans have been shown to have wider spectrum and higher intensity of parasites and higher prevalence of antibiotic resistant bacteria compared to their counterparts with little human contact (Rolland et al., 1985).

The number of gastro-intestinal parasites which are widely shared between human and NHPs include: *Strongyloides fulleborni, Trichuris trichiura, Oesophagostomum sp.*, *Trichostrongylus sp.*, *Enterobius vermicularis, Schistosoma mansoni, Ascaris sp*, *Entamoeba histolytica, Giardia sp, Isospora sp.*, *Blastocystis sp.* and *Balantidium coli*. Although these parasites have been reported with very high prevalence in NHPs in Kenya and other African countries, these studies are mainly based on results obtained from NHPs caught for biomedical research (Munene et al., 1998). Proper epidemiological studies on these parasites are, however, lacking. Due to the myriad variables that influence pathogen transmission, single academic discipline and sectors are inadequate in researching and controlling the zoonoses. Rather, a “One Health”
approach has been advocated where collaborative efforts of anthropologists, primatologists, veterinarians, physicians, epidemiologists, microbiologists and virologists, is integral to understanding the complex interplay of factors that are involved in human–NHP pathogen transmission (Travis et al., 2006). The objective of the present study was to determine the zoonotic GIT helminths and haemoparasites and the risk factors associated with transmission of these zoonotic parasites.

1.2 Statement of the problem

There has been an increase in the human population in the country which has led to a need for increased food production and also more land for habitation. This has led to humans encroaching into wildlife land (Kivai, 2010). This therefore brings about a human-wildlife conflict, with humans feeling they have more rights to land than animals. Humans inhabiting park borders also need to farm as that is their main source of livelihood. However, with the climatic changes which have caused loss of vegetation, wildlife and especially baboon raid farms in search of food (Butynski and Mwangi, 1994). This may lead to increased human–wildlife interaction and zoonoses. Currently there is no data in Kenya on prevalence of zoonotic helminths and haemoparasites and the risk factors associated with their transmission. Studies carried out on these parasites have been done in captive baboons mainly in helminthes (Munene et al., 1998; Muriuki et al., 1998; Farah et al., 2005). There is no integrated data on both helminths and haemoparasites and data on hematology responses is also lacking in these animals in the country. Data on pathological responses associated with parasitic infections in baboons
is scanty and data is also lacking on risk factors associated with zoonoses studied from the same site as baboon zoonotic parasites.

1.3 Study justification

Currently information on zoonotic helminths and haemoparasites in baboons in Kenya is limited. As earlier indicated, previous studies have only dwelt on specific areas in isolation. Most of them have also been mainly based on captive baboons. This is not realistic in some instances since the physiology of animals change once in captivity and may not provide the same information as the ones in the wild. Baboons are major pests and interact more with humans compared to other wildlife. Diseases such as Babesia microti and soil transmitted helminths are zoonotic and can be asymptomatic in baboons but cause severe infection in humans. The current study sort to provide information on zoonotic helminthes and haemoparasites in baboons and the risk factors associated with interaction of humans and baboons at human wildlife interface. Three study sites identified for the study were Mutara ranch in Laikipia, border of Tsavo West National Park and Tana River Primate National Reserve. The sites were selected to provide a wider picture of the country since Laikipia is found in Central Kenya, Tsavo is in the Eastern part of the country while Tana River is found in the coastal region. Each of the sites is also unique because in Mutara Ranch, wildlife freely roam around and interact with humans. In Tsavo West National Park, there is an attempt to confine wildlife inside the park with fencing while in Tana River Primate National Reserve, people are allowed to farm in the reserve and are expected to assist in conservation. These sites therefore
give three different levels of human baboon interaction. The baboons were selected because of their close physiology to humans (Farah et al., 2009) hence chances of harboring zoonotic pathogens which have also been reported.

Prevalence and intensity of parasites was important to determine the level of infection of these baboons. This was the only way of knowing presence of zoonotic parasites in these baboons. Questionnaire survey was also carried out within the same sites of baboon studies to determine the level of interaction and find out if there were chances of zoonotic transmission. This is because it may just be imagined that with humans and baboons being close then automatically there is zoonotic transmission but this had to be confirmed. Zoonotic diseases are a major challenge currently with most emerging diseases coming from animals. With presumed high level of interaction between baboons and humans, these zoonotic pathogens are likely to be passed to humans from baboons.

1.4 Research questions

i) What are the prevalences of zoonotic gastrointestinal helminths in baboons from Tsavo, Tana River and Mutara Ranch, Laikipia County?

ii) What are the prevalences of haemoparasites in baboons from Tsavo, Tana River and Mutara ranch, Laikipia County?

iii) What are the leucocyte response following helminth treatment in baboons?

iv) What are the pathological effects of gastrointestinal zoonotic helminths in
v) What are the risk factors associated with the transmission of zoonoses at baboon-human interface?

1.5 Hypothesis

Baboons found in Tana River, Tsavo and Mutara Ranch, Laikipia County do not harbor zoonotic haemoparasites and gastrointestinal helminths hence no pathology associated with helminths in the baboons and no risk factors associated with zoonotic transmission in the three study sites.

1.6 Objectives

1.6.1 General objective

To investigate zoonotic haemoparasites and gastrointestinal helminths affecting baboons and the pathology they cause and risk factors associated with their transmission in Tana River, Tsavo and Mutara ranch in Laikipia County.

1.6.2 Specific objectives

i. To determine the prevalence of zoonotic gastrointestinal helminthes in baboons in Tsavo National Park, Tana River Primate Reserve and Mutara ranch, Laikipia.

ii. To determine the prevalence of zoonotic haemoparasites in baboons in Tsavo National Park, Tana River Primate Reserve and Mutara ranch, Laikipia.

iii. To determine the response of leucocytes following helminth treatment in
baboons

iv. To determine the pathology associated with zoonotic gastrointestinal helminths in the baboons.

v. To determine the risk factors associated with transmission of the zoonoses at the baboon-human interface.

1.7 Significance of the study

The study provides information on the zoonotic parasites existing in baboons, hence a database of zoonotic pathogens in Tana River, Tsavo National Park areas and Mutara ranch in Laikipia County. This is important information that can be used in control strategies of the infections. Without public awareness on zoonosis from baboons, there will be a cycle of re-infections even with good treatment strategies in place which may also lead to drug resistance. The study also avails information to stakeholders on the human–baboon conflict which need to be resolved for continued coexistence between the two. This is important for continued preservation of the animal as a wildlife attraction.
CHAPTER TWO: LITERATURE REVIEW

2.1 Human-wildlife interactions

With increasing population and pressure on forest areas, human-wildlife interaction and resultant conflict is also increasing (Zaire and Switzer, 2001). Human-wildlife conflict is defined as "any interaction between humans and wildlife that results in negative impacts on human social, economic or cultural life, on the conservation of wildlife populations, or on the environment (WWF, 2005). In the past, rural residents, especially agricultural producers and forestry owners suffered most from wildlife damage but this has changed more recently as urban residents and other wildlife stakeholders are increasingly experiencing the problem (Messmer, 2000; Distefano, 2005).

The phrases ‘animal damage control’, ‘problem wildlife management’, and ‘wildlife damage management’ have traditionally been used to describe actions taken to reduce economic losses to agricultural produce caused by wildlife. More recently, the phrase ‘human–wildlife conflict management’ is being applied to these and other situations that involve any negative interactions between humans and wildlife (Messmer, 2000). These conflicts can be real or perceived, economic or aesthetic, social or political. Among the losses incurred during the conflict include: raiding and destruction of food crops, loss of income from sales of produce from cash crops, damage to water sources and installations, damage to stored produce, loss of livestock, human injury or death and damage to property such as buildings (Woodroffe et al., 2005; WWF, 2005; West et al., 2006). Among the contributors of conflict are crop raid and livestock predation. Crop-

raiding animals may cause substantial damage to agricultural crops, and this has always been a major issue of contention throughout the world.

Due to the expansion of cultivated land into previous wildlife habitat, crop raiding is becoming one of the most common conflicts antagonizing human-wildlife relationships. The most notorious crop raiders are wild boars, elephants, birds, and various ungulates (Badola, 1998; Zubiri and Switzer, 2001; Johnsingh and Negi, 2003; WII 2005) and non-human primates. Livestock are often preyed upon by wild carnivores, which often lead to the persecution of the perceived killers. This is more common with predators such as the large cats, wolves and tigers in various parts of the world inhabiting the same area or live close to areas used by livestock (Zubiri and Switzer, 2001; West et al., 2006). Compensation is a major challenge and in hard to come by in most countries and in most cases is marred with corruption and the process of verification takes very long in some cases as is the case in India (Ogra and Badola, 2008). Botswana is the only member of the Southern African Development Community (SADC) to employ a state funded compensation system. Compensation systems are based upon paying reparations to property owners for losses incurred due to wildlife (Hemson, 2004). The underlying tenet of all compensation schemes is that payments encourage tolerance for losses by minimizing the economic impact of these losses (Nyhus et al., 2003).

Wildlife perceived as a problem are killed either by individuals, hunters, or by the governments and methods used include shooting, trapping and poisoning (Woodroffe et
Peoples’ attitudes towards wildlife are complex, with social factors as diverse as religious affiliation, ethnicity and cultural beliefs all shaping conflict intensity. Moreover, human–wildlife conflicts are often manifestations of underlying human–human conflicts, such as between authorities and local people, or between people of different cultural backgrounds (Dickman, 2010). Despite evidence that social factors can be more important in driving conflict than wildlife damage incurred, they are often ignored in conflict studies. Developing a broader awareness of conflict drivers will advance understanding of the patterns and underlying processes behind this critical conservation issue (Dickman, 2010). A successful resolution of human-wildlife conflict requires the participation of local communities and other stakeholder groups in formulating management decisions (Redpath et al., 2004).

2.2 Zoonotic diseases

2.2.1 Wildlife-Human zoonotic diseases

Wildlife diseases are a major concern to humans since most of them can be transmitted to domestic animals (Gortazar et al., 2007). A number of these diseases are transmitted to other hosts such as humans, domestic and wild carnivores when they feed on these animals (Rizzoli and Bryan, 2002). Some pathogens do exclusively infect a single host species (Gortazar et al., 2007) while others infect various hosts and others are zoonotic. Human diseases of animal origin (zoonoses) comprise more than 150 infections of various etiologies. They also form a sizeable proportion of the new, emerging and re-emerging diseases (Schwabe, 1984; Acha and Szyfres, 1989; Meslin, 1995).
The zoonotic introduction of an animal pathogen into the human population and the subsequent extension or alteration of its host range leading to the successful maintenance of the corresponding pathogen by human-to-human transmission pose a serious risk for world-wide health care (Bastone et al., 2003). The occurrence of wide spectrum of zoonoses in NHPs, which make them a leading source of emerging and re-emerging diseases, has become a major challenge for the public and international health systems and a scientific preparedness is a prerequisite to finding appropriate solutions for these threats (Wolfe et al., 2005). An analysis of emerging infectious diseases (EID) between 1940 and 2004 demonstrated a marked increase in the number of EID, the majority of which were of zoonotic origin from wildlife, and less of vector-borne origin. The study also showed that EID events are significantly associated with socioeconomic and ecological factors where regions that have lower income and in lower latitudes has a higher probability of experiencing an EID event (Jones et al., 2008).

The human AIDS viruses, human immunodeficiency virus type 1 (HIV-1) and type 2 (HIV-2) are known to be zoonotic (Hirsch et al., 1989; Huet et al., 1990; Sharp et al., 1995). The primate reservoir for HIV-2 has been reported to be the sooty mangabeys (Cercocebus atys) whereas that of HIV-1 is the Chimpanzee (Hirsh et al., 1989). Two chimpanzee subspecies in Africa, the central P. t. troglodytes and the eastern P. t. schweinfurthii, are reported to harbor SIVcpz which is related to HIV-1 virus (Gao et al., 1999).
Ebola outbreak in West Africa in 2014 was one of the most severe zoonoses which by March 2015 had over 24,000 cases and almost 10,000 mortalities had been reported in an outbreak that occurred in 2014 in West Africa (CDC, 2015). A study by Leroy et al. (2004) indicated that several human and animal Ebola outbreaks had occurred in the previous 4 years in Gabon and the Republic of Congo. The human outbreaks consisted of multiple simultaneous epidemics caused by different viral strains, and each epidemic resulted from the handling of a distinct gorilla, chimpanzee, or duiker carcass (Leroy et al., 2004). These animal populations declined markedly during human Ebola outbreaks, apparently as a result of Ebola infection. Recovered carcasses were infected by a variety of Ebola strains, suggesting that Ebola outbreaks in great apes result from multiple virus introductions from the natural host.

These pathogens therefore are not just virulent to humans but also to the animal hosts. Previous studies done in other primates such as chimpanzee populations have shown high rates of mortality in wild chimpanzee populations associated with anthropozoonotic transmission of human respiratory viruses (Kondgen et al., 2008) and the natural threats of Ebola, anthrax and SIV (Leroy et al., 2004; Leendertz et al., 2006; Keele et al., 2009). The human Ebola outbreaks previously consisted of multiple simultaneous epidemics caused by different viral strains, and each epidemic resulted from the handling of a distinct gorilla, chimpanzee, or duiker carcass.

Chimpanzees, humans’ closest living relatives are not only genetically similar to
humans but also share susceptibility to some infectious diseases. For instance, outbreaks of haemorrhagic fever in humans caused by relatives of Ebola virus in chimpanzees and gorillas have resulted in marked mortality in wild populations (Robin et al., 2009). In addition to the threat of AIDS and Ebola, the NHPs may also be at risk of acquiring other infections from humans from their increasingly close contact (Robin et al., 2009).

The Foamy Viruses (FV) of wild-ranging and captive nonhuman primates and the simian FVs (SFV) have repeatedly been shown to cross host-range barriers resulting in zoonotic transmission to humans (Schweizer et al., 1997; Heneine et al., 1998). Feline foamy virus (FFV) requires direct contact with infected cats for transmission. Since the virus can be recovered from the mouth of infected cats (Alke et al., 2000), cat bites are also a way of transmission of FFV by breaking the intact skin barrier and allowing access to susceptible cells, for instance, leukocytes (Bastone et al., 2003). In the developed countries, exposure of humans to NHP and the risk of zoonotic SFV transmission is almost entirely restricted to few laboratory workers and animal (NHP) caretakers. In contrast, in these countries, exposure to the pathogens of companion animals or livestock is much more likely and potentially affects almost the whole population.

The zoonotic risk arises either by contacting feline foamy virus (FFV), bovine foamy virus (BFV) and equine foamy virus (EFV) infected animals or, alternatively, by food or medical products directly or indirectly derived from the FV-infected hosts (Bastone,
The occurrence of zoonotic transmissions of animal viruses to humans is a permanent threat to human health and is even increased by changes in the human lifestyle (Bastone et al., 2003). The risk of zoonotic transmission directly increases with the genetic relatedness of the authentic and novel host (Saib, 2003).

The exchange of disease is a concern for wildlife conservation both outside and inside the boundaries of parks and reserves. Lands adjacent to parks are increasingly being converted to residence hence being used for crop cultivation and livestock domestication. In addition, the presence of tourists, researchers and park personnel have created a situation that may facilitate disease transmission between humans, livestock and wildlife (Simonetti, 1995). The Tana River National Primate Reserve is a key primate ecosystem in Kenya and is home to many primate species, including endangered species. The human-non human primate conflict is mediated by rapidly shrinking primate habitat. The forest fragmentation restricts the primates in small area and subsequent decline of their food resources (Kivai, 2010). Previous studies have shown that hunting for bush meat is carried out in primate populations in different habitats (Butynski, 1985; Mittermeier, 1987; Moinde et al., 2004). Changes in socio-economic status and the concentration of humans in urban environments were shown in Eastern Europe to lead to an increase in the incidence of tick-borne encephalitis (TBE) cases (Sumilo et al., 2008). There are reports that although an increase in infected wildlife or Ixodes ticks was not necessarily detected, other societal changes (change in land-use, reduced amount of pesticide usage, and an increase in human density) were
factors responsible for bringing humans into closer contact with infected ticks (Sumilo et al., 2008).

2.2.2 Gastrointestinal tract (GIT) helminths

Helminth infections are parasitic worms found in the intestinal tract, urinary tract or blood of humans (Brooker et al., 2009). The helminth species that cause the greatest human morbidity are the schistosomes, intestinal nematodes (or commonly called soil-transmitted helminthes, STH), and tissue nematodes, including human filariae that cause lymphatic filariasis and onchocerciasis (Muller, 2002). Although helminth infections can infect all members of a population, it is clear that there are specific groups who are at greater risk of morbidity than others, and who are more vulnerable to the harmful effects of chronic infections (Hotez et al., 2006; Brooker and Bundy, 2008). For schistosomes and STH, the most vulnerable groups are school-aged children and women of child-bearing age, including adolescent girls (Crompton and Nesheim, 2002; Christian et al., 2004; Brooker et al., 2009). These infections are the so called neglected tropical diseases (NTDs), infections which were initially thought to be of less significance but have now drawn the world’s attention and various measures are being put in place to reduce their prevalence or eliminate them (Brooker et al., 2009).

Nearly one-third of the global population is infected with helminth parasites, rendering them among the most prevalent infectious agents in the world today, and they are responsible for many debilitating diseases and syndromes (Hotez, 2008). Soil-
transmitted helminthiasis remains an important cause of morbidity and sometimes mortality in developing tropical countries, particularly among pediatric age group (WHO, 1987). It is estimated that more than one billion people in the world are infected by soil-transmitted helminths (STH), mainly Ascaris lumbricoides, Ancylostomum americana and Trichuris trichiura (Crompton, 1999). Although STH affect all age groups, the problem is predominant among the world’s estimated 400 million school children, and is often associated with poor growth, reduced physical activity, impaired cognitive function and learning ability (Stephenson et al., 1998). Infections with GIT parasites are widespread among non-human primates (NHPs). However, as a consequence of regular deworming and hygienic measures, helminth infections are uncommon in captive NHPs (Gomez et al., 1996) and no such programmes exist for free ranging NHPs and other wildlife.

In contrast, protozoa such as Entamoeba histolytica, Giardia spp., Cryptosporidium spp. (Gomez et al., 1992) and Balantidium coli are frequently reported in captive NHP, and are considered as important causes of gastro-enteritis in NHP. Infection by these GIT parasites may cause watery diarrhea, hemorrhagic dysentery, extra-intestinal pathologies, such as liver abscesses, and even death. Entamoeba histolytica, the causative organism of invasive intestinal and extra-intestinal amoebiasis, is of major importance in humans. Study conducted by Munene et al. (1998) on NHPs identified the following zoonotic parasites in Kenya’s free ranging baboons; Strongyloides fulleborni, Trichuris trichiura, Oesophagostomum sp., Trichostrongylus sp., Enterobius
vermicularis, Schistosoma mansoni, Ascaris sp, Entamoeba histolytica, Giardia sp, Isospora sp., Blastocystis sp. and Balantidium coli.

Similarly, a study conducted in six non-human primates in Kenya namely olive baboons (Papio cyanocephalus anubis), Vervet monkey (Chlorocebus aethiops), Sykes monkey (Cercopithecus mitis), Black and white colobus (Colobus abyssinicus), Debrazzas monkey (Cercopithecus neglectus) and Grey and Black mangabeys (Cercocebus torquatus and Cercocebus albigena) indicated several zoonotic parasites in these primates, with the baboon being the most implicated (Muriuki et al., 1998). Trichuris sp. was the most frequent helminth followed by Strongyloides fulleborni, Strongyles sp. and Schistosoma mansoni, in that order. Among the GIT protozoal infections, Entamoeba coli was the most common, followed by Balantidium coli and Entamoeba histolytica respectively. All primate species examined were infected with all the parasites listed except the black and white colobus. Cryptosporidium spp. were found in both clinically normal and diarrhoeic baboons and vervets (Muriuki et al., 1998).

Schistosome and soil-transmitted helminth (STH) infections cause a huge burden of disease in the developing world (Chitsulo et al., 2000) and have been associated with significant educational and nutritional effects (Stephenson, 1993; King et al., 2005). School age children suffer from subtle morbidity such as anemia, chronic pain, diarrhea, exercise intolerance, growth stunting, under-nutrition and impaired cognitive development, leading to poor school performance (Miguel and Kremer, 2003; King et
In children and adults, *Schistosoma mansoni* can have nonspecific clinical manifestations such as bloody diarrhea and abdominal discomfort, and if untreated can lead to serious liver complications and poor growth. During pregnancy, hookworms and schistosomiasis have been reported to cause neonatal prematurity, reduced neonatal birth weight, and increased maternal morbidity and mortality. While this burden is difficult to measure, deaths due to schistosomiasis have been estimated as high as 200,000 per year worldwide (Christian et al., 2004; King et al., 2005).

In Kenya, over 6 million people are estimated to be infected with helminthes and many more are at risk (Chitsulo et al., 2000). The highest infection rates are found in adolescents aged 10–19 years, but adult workers in rural areas who are employed in activities associated with water contact are also affected (Karanja et al., 1997; Karanja et al., 1998). Overall, the prevalence of schistosomiasis ranges from 5% to over 65% in communities in Kenya and contributes to significant morbidity (Ouma et al., 2001; Mwinzi et al., 2004). In the Nyanza region, schistosomiasis is largely associated with Lake Victoria (Handzel et al., 2003). There are still many areas where the true burden with schistosomiasis is not well known as a disease (Mwinzi et al., 2012). The relatively wide distribution of hookworm is apparent in most surveyed areas in East Africa, except in northern Kenya and northeast Uganda. The distribution of *A. lumbricoides* and *T. trichiura* infection is more restricted, with high prevalence estimates reported in Burundi and Rwanda, central and western Kenya, southeast
Uganda, northeast Tanzania and Zanzibar. *Schistosoma mansoni* infection was reported to be most prevalent around Lake Victoria basin, North-west Uganda and the central highlands of Kenya. In contrast, *S. haematobium* infection was distributed along the Kenyan and Tanzanian coast, Tana River in Kenya and Lake Victoria in Kenya and Tanzania, but absent in Uganda (Brooker et al., 2009) (Figure 2.1 and 2.2).
Figure 2.1: Geographical distribution of Soil Transmitted Helminths in East Africa. (A) hookworm, (B) *Ascaris lumbricoides* and (C) *Trichuris trichiura* (Brooker et al., 2009)

![Map of East Africa showing distribution of helminths](image)

Figure 2.2: Geographical distribution of schistosomiasis in East Africa. (a) *Schistosoma haematobium* and (b) *S. mansoni* infection (Brooker et al., 2009)

In addition to children being the most affected, they also tend to both harbor the greatest number of worms and be most susceptible to their effects, which include anaemia, growth stunting, and reduced physical fitness, educational performance and school attendance (Crompton and Nesheim, 2002). Though death is an uncommon outcome, this group of infections has enormous poverty-promoting impact (Hotez and Ferris, 2006). The most important STHs are the common roundworm (*Ascaris lumbricoides*), the whipworm (*Trichuris trichiura*) and the hookworms (*Necator americanus* and *Ancylostoma duodenale*). The greatest burden of disease is found in the Americas,
China and Sub-Saharan Africa. These worms have similar lifecycles in that adults infect the gastro-intestinal tract, reproduce sexually and release eggs which are passed in faeces into the environment. Hookworms differ from *Ascaris* and *Trichuris* in that, rather than being acquired through ingestion of eggs, larvae develop to the infective stage in the soil then penetrate intact skin to initiate the parasitic phase (Hotez *et al*., 2008).

Some members of populations in which STHs are endemic are more ‘wormy’ than others: commonly 70% of the community worm burden is concentrated in 15–30% of the population (Albonico *et al*., 1999). The role of protective immunity in creating this ‘aggregated’ or ‘over dispersed’ distribution remains poorly understood. At the community level, WHO use both prevalence and intensity of infection to categorize communities into high, medium and low transmission intensity environments, in order to inform mass treatment strategies (Montresor *et al*., 1998).

Five species of parasitic trematodes of the family Schistosomatidae infect humans. They have a complex life cycle involving snails as intermediate hosts. Infected snails shed cercariae into the water, which can penetrate intact human skin and locate and enter post-capillary venules (Feasey *et al*., 2010). Further development takes place in the lungs and liver before migration to the perivascular venous plexus (*Schistosoma haematobium*) or the mesenteric veins (*S. mansoni, S. japonicum, S. intercalatum* and *S. mekongi*). Adult pairs remain in copula at these intravascular sites for the remainder of their lives. Acute illness (‘Katayama fever’) is characterized by fever, lethargy and
eosinophilia, occurring a few weeks after first infection (Doherty et al., 1996). Most of the disease burden from schistosomiasis (bilharzias), however, is caused by passage of eggs through the walls of blood vessels and intestine, ureters or bladder, or by eggs lodging in liver or lungs then washed away by the portal venous system. Over time, the resulting granulomatous inflammation can lead to haematuria, bladder obstruction, renal failure or bladder cancer in *S. haematobium* infection, or, in infection with mesenteric schistosomes, periportal fibrosis, portal hypertension, ascites and varices in *S. mansoni* infection. Eggs that reach the lumen of bladder or bowel are expelled in urine or faeces. Contained miracidia are released when the egg is immersed in water, and actively seek out and penetrate the snail intermediate host (Gryseels et al., 2006). Diagnosis of chronic disease is by the detection of eggs in urine or faeces, usually after concentration methods have been applied. Serological diagnosis is sensitive but non-specific (Gryseels et al., 2006).

*Oesophagostomum* - another gastrointestinal helminth is characterized by gross nodule formation in the intestinal mucosa (Lapage, 1962; Soulsby, 1965; Smith et al., 1992). The larvae penetrate the mucosa at any point from the pylorus to the anus in order to reach the deeper parts of the sub-mucosa where they encyst and undergo molting. Local tissue sensitivity develops in animals due to repeated exposure to these parasites and the subsequent entry of the larvae into the submucosae which provokes an intense tissue reaction. The parasites produce glandular secretions which are responsible for the chronic inflammation in the intestinal wall resulting in tissues fibrosis (Lapage, 1962;
Smith et al., 1992). Microscopically, its pathology has been characterized by catarrhal inflammation associated with destruction and desquamation of epithelial cells in goats (Babu et al., 2011).

Helminth parasites have developed complex strategies to modulate the immune responses of their hosts through utilizing versatile immunoregulatory mechanisms to avoid immune effector cells and molecules. In both human and animal hosts, helminths establish long-term chronic infections associated with significant degrees of down regulation of the host immune response (Maizels and Yazdanbakhsh, 2003; Hoerauf et al., 2005; Elliott et al., 2007). These parasites are associated with Th2 responses with elevation of IL-4, IL-5, IL-9, IL-10 and IL-13. It is also reported that these infections compromise immunity to other unrelated infections and may affect the efficacy of vaccines (Allen and Maizels, 2011). Various cell populations are affected by helminth infections, including macrophages, dendritic cells (DCs), T regulatory cells (Treg), mast cells, and neutrophils (Mylonas et al., 2013). Thus, helminths use multiple means to escape or modulate the immune response in their hosts (Maizels et al., 2004). There is also evidence that the immunomodulatory activities of helminthes could impact on outcome of several inflammatory diseases, such as multiple sclerosis (MS), arthritis, type 1 diabetes (T1D), and inflammatory bowel diseases (IBD) including ulcerative colitis and Crohn’s disease (Elliott and Weinstock, 2012).
2.2.3 Haemoparasites

Babesiosis is a tick-transmitted disease caused by intraerythrocytic parasites of the genus *Babesia*. Babesial parasites are capable of infecting a wide variety of mammals, and awareness is growing of their role as zoonotic agent of human disease (Homer *et al*., 2000). Babesia-like parasites of the genus *Entopolypoides macaci* have been reported to infect nonhuman primates; based on phylogenetic analysis of small-subunit rRNA (SSUrRNA) sequences of this parasite and on serological and epidemiological data. It was suggested that the genus *Entopolypoides* is synonymous with that of *Babesia* (Bronsdon *et al*., 1999). In primate centers, natural infections with this parasite have been shown in baboons (*Papio cynocephalus*) (Bronsdon *et al*., 1999) long-tailed macaques (*Macaca fascicularis*), (Emerson *et al*., 1993) and rhesus macaques (*Macaca mulatta*), providing animal models in species closely related to humans to study parasite–host relationships of this pathogen (Voorberg-vd Wel *et al*., 2008).

Human babesiosis is an important emerging tick-borne disease. *Babesia divergens*, a parasite of cattle, has been implicated as the most common agent of human babesiosis in Europe, causing severe disease in splenectomized individuals. In the US, *Babesia microti*, a babesial parasite of small mammals, has been the cause of over 300 cases of human babesiosis since 1969, resulting in mild to severe disease, even in non-splenectomised patients. This parasite is closely related to babesial parasites isolated from large wild ungulates in California (Kjemtrup and Conrad, 2000). There have been reports of *Babesia microti* infection in Kenyan free ranging baboons (Mamuun *et al*.,...
Phylogenetic analysis of nuclear small-subunit rRNA gene sequences amplified from peripheral blood of a baboon chronically infected with *E. macaci* demonstrated this parasite to be most closely related to *Babesia microti* (97.9% sequence similarity); sera from infected animals did not react in indirect fluorescent-antibody tests with *Babesia microti* antigen suggesting that they represent different species (Bronsdon *et al.*, 1999).

The presence of an under recognized, but highly enzootic, *Babesia* sp. in baboons may result in substantial, unanticipated impact on research programs. The similarity of this parasite to the known human pathogen *B. microti* may also pose risks to humans undergoing xenotransplantation, mandating effective screening of donor animals (Bronson *et al.* 1999). Anaplasmosis is another haemoparasite that is zoonotic and in humans it is known as human granulocytic anaplasmosis (HGA) (previously known as Human granulocytic ehrlichiosis (HE)). This is an infectious disease caused by *Anaplasma phagocytophilum*, an obligate intracellular parasite that is typically transmitted to humans by at least three kinds of ticks, including *Ixodes scapularis*, *Ixodes pacificus*, and *Dermacentor variabilis* (Malik *et al.*, 2005). The disease presents with fever, chills, severe headache and myalgia. Over 77 million dogs and 93 million cats are reported to share households in the United States.

Multiple studies have demonstrated the importance of pets in their owners' physical and mental health (Esch and Petersen, 2013). Given the large number of companion animals
in the United States and the proximity and bond of these animals with their owners, understanding and preventing the diseases that these companions bring with them are of paramount importance. Zoonotic protozoal parasites, including toxoplasmosis, Chagas' disease, babesiosis, giardiasis, and leishmaniasis, can cause insidious infections, with asymptomatic animals being capable of transmitting disease (Esch and Petersen, 2013).

Giardia and *Toxoplasma gondii*, endemic to the United States, have high prevalences in companion animals. *Leishmania* and *Trypanosoma cruzi* are found regionally within the United States. These diseases have lower prevalences but are significant sources of human disease globally and are expanding their companion animal distribution. Thankfully, healthy individuals in the United States are protected by intact immune systems and bolstered by good nutrition, sanitation, and hygiene. Immunocompromised individuals, including the growing number of obese and/or diabetic people, are at a much higher risk of developing zoonoses. Awareness of these often neglected diseases in all health communities is important for protecting pets and owners (Esch and Petersen, 2013).

### 2.3 Control of zoonotic diseases

#### 2.3.1 Health education

Health education is very vital in disease control strategies since knowledge is deemed to give power to the individual and reduce the level of ignorance. There is need for higher level of authority especially the government to take a lead role in educating its citizens
on disease control strategies (Jamison et al., 2006). This is because the resources needed in such endeavors can be more and as such education should be continuously done. Apart from community training, it has also been incorporated in school syllabus even at elementary levels in various countries (CDC, 2015). Health education is very important in control of communicable diseases. It is also pointed out to be a riding tool especially for helminth infections to implement other key control measures such as chemotherapy and sanitation (Asaolu and Ofoezie, 2003). In the developing countries, this needs to be scaled up as the problem for infectious diseases is still a challenge (Jamison et al., 2006).

### 2.3.2 Environmental management

Environmental management is vital in control of disease especially vector control. By modification of the environment, various diseases can be controlled as indicated by Ault (1993). These management strategies consist of permanent or long-term modification of the environment, temporary or seasonal manipulation of the environment, and modifying or changing human life styles and practices to reduce human contact with infective vectors (Ault, 1993). Specifically these activities include suppression of vector populations through the provision of safe water supplies, proper sanitation, solid waste management facilities, sewerage and excreta disposal systems, water manipulation in dams and irrigation systems, vector diversion by zoo prophylaxis, and vector exclusion by improved housing. These environmental management can also be integrated with pharmacological, insecticidal and bed net interventions for control of various infections.
especially mosquito transmitted (Utzinger, 2001).

Vector-borne diseases are reported to account for approximately 17% of the estimated global burden of infectious disease (WHO, 2004) most of which are zoonotic. Vector control has been pointed out as a very effective tool in control of disease transmission (Townson et al., 2005). In malaria for instance, use of insecticide-treated bed nets and indoor spraying of houses with insecticides have been reported to be very effective in preventing malaria transmission, translating into reduced morbidity and mortality (Curtis and Davis, 200; Lengeler, 2004). There are diseases such as dengue which is also zoonotic and Chagas disease whose only control measure available in humans is vector control (Schofield and Dias, 1999; WHO, 2002). This method can also be the most cost-effective option when unit costs of individual case detection and treatment become progressively greater as case numbers drop especially in cases where it is used together with chemotherapy (Townson et al., 2005). This can be done in a multidisciplinary approach involving pesticide-based control or involving environmental modification, in addition to a strengthened managerial and operational capacity. When implementing these approaches several factors should be considered for better outcomes. These are determinants of disease transmission, including local disease ecology, the role of human activity in increasing risks of disease transmission, and the socioeconomic conditions of affected communities (Townson et al., 2005).
2.3.3 Preventive chemotherapy (PCT)

For most helminthic infections, there is still a challenge in development of vaccines and chemotherapy still remains the best option in controlling these infections. Sanitation on the other hand helps to sustain the benefits of chemotherapy and also in protecting the uninfected (Asaolu and Ofoezie, 2003). Control for most of the helminths currently is by use of anthelminthic drugs a method popularly known as preventive chemotherapy (PCT) (Taylor-Robinson, 2007; Keiser and Utzinger, 2008). For schistosomiasis, snail control with molluscides is complicated, poorly cost-effective and potentially toxic to other water life (Gryseels et al., 2006). Recommended methods of control include mass distribution of praziquantel, improved access to safe water and sanitation and health education (WHO, 2002; Savioli et al., 2004). In its first 5 years of operation from 2003 to 2008, Schistosomiasis Control Initiative-supported programmes have administered praziquantel to more than 44 million people in six countries in Africa (Feasey et al., 2010).

In Africa, an increasing number of countries are implementing national treatment programmes for the control of soil-transmitted helminths (STH) (Pullan et al., 2011). The main strategy of these programmes is the delivery of deworming through the public school system, which has been demonstrated as a cost-effective way to reduce infection and morbidity of STH and improve educational outcomes (Guyatt et al., 2001; Brooker et al., 2008). There have also been moves to integrate mass drug administration (MDA) for STH and schistosomiasis with other neglected tropical diseases (NTDs), including
lymphatic filariasis (LF) and onchocerciasis (Hopkins et al., 2002, Ndyomugenyi and Kabatereine, 2003). For STH, the benzimidazole anthelmintics, albendazole and mebendazole, are the treatments of choice (Hotez et al., 2008). Nematodes in livestock develop resistance to these drugs when they are used repeatedly, and there is concern that this may account for the decreased efficacy of human mass treatment noted in some settings (Geerts and Gryseels, 2000).

2.4 Gaps in knowledge

Based on the above literature, it is clear that there are many challenges when dealing with wildlife zoonosis, among this, being limited information on wildlife diseases (Gortazar et al., 2007). Thus, more experimental approaches are needed to produce substantial knowledge that enables authorities to put in place successful control programmes (Gortazar et al., 2007). There is also an upward trend in urbanization which even poses a higher threat of wildlife diseases especially from non-human primates more so baboon due to their close interaction with humans in such settings. With the urbanization, there are increased risks of multi-host pathogens for humans and vulnerable wildlife populations and therefore, the need for more studies of wildlife diseases especially in such settings (Bradly and Altizer, 2007). The current study sought to investigate baboons zoonotic infections, animals that have high interactions with humans both in the urban and rural settings and the information gained in the current study will shed more light on not only on zoonotic geohelminths and hemoparasites in the animals, but also the risk factors that enhance the disease transmission to humans.
Although there is clear knowledge that baboons do harbor zoonotic parasites – both hemo and GIT helminthes, this varies with the location and also with the level of human–NHPs interaction. In spite of possible emergence of zoonoses transmission between NHPs and humans, the frequency of transmission of such infections in wild primates having human contact remains un-quantified and organized efforts to prevent disease transmission are lacking (Baruch-Mordo et al., 2009). This is more so in Kenya where most studies have been carried out in specific aspects in baboons mainly helminthes and in most cases on captive baboons. It is rare to find an individual with one disease therefore studying more than one infection in the baboons gives a better picture of the pathogens if not all. Such studies had not been carried out in wild baboons. The current study focused on the prevalence and intensity of these parasites in three locations in Kenya which also provides a broader picture of pathogens in the Kenyan baboons as compared to previous studies which have been mostly limited to one site.

There are various studies on pathology of helminthes in baboons but most of them have been experimental hence the current study aimed to provide information on pathology of helminthes and hemoparasites on naturally infected baboons, information which was lacking. Studies incorporating zoonotic pathogens screening and risk factors associated with their transmission was also lacking which the current study also dwelt on. These are the aspects that the current study focused on in three sites of Kenya namely; Tsavo west, Tana River and Mutara ranch in Laikipia County.
CHAPTER THREE: MATERIALS AND METHODS

3.1 Study area

The study areas included Tana River Primate National Reserve (TRPNR), Tsavo West National Park (TWNP) and Mutara ranch in Laikipia (Figure 3.1).
Figure 3.1: Map indicating three study sites for baboon sampling (Google, 2015)
(i) Mutara Ranch, Laikipia (ii) Tana River National Primate reserve and Tsavo National park
In these areas, humans closely interact with baboons. Tana River Primate National Reserve is a non-human primate (NHPs) conservation site where humans and NHPs share the habitat and a common water source- River Tana. The forest is a good habitat for NHPs while humans farm along the river bank and also use the water for domestic activities and watering livestock. Tana River flows in the Lower Tana River gallery forest which is one of the rare yet complex habitats in eastern Africa. The lower flood plain of the Tana River, the largest river in Kenya contains a unique community of riverine forest vegetation in scattered patches of various sizes. The forest is a relic of a previously more continuous rainforest that extended from the Congo River Basin to the east coast of Africa during the Pleistocene era. Later, prolonged droughts led to the shrinkage and isolation of east African evergreen forests, leaving them confined to moist highland and riverine areas (KWS, 2013). The forest is unique because it supports a high diversity of plant and animal species. It is about 100 km upstream from the Tana delta, between latitudes 2°15’ and 1°50’ south (Tana River County, 2015).

Tsavo West National Park was selected because there are human habitations around the park that practise both livestock and crop cultivation and baboons are also ubiquitous in the park. The site consists mainly of semi-arid plains, granite outcrops and ancient lava fields. For most of the year, Tsavo is dry and dusty. Generally, the weather in Tsavo is warm and dry, with temperatures ranging from 20°C-40°C and rainfall from 200-700mm per year. There are two rainy seasons; the long rains are generally from March through to May when the weather is hot and humid while the short rains come in the
warm months of October to December (FAO, 2015, Fallingrain, 2015). The study areas in this site was the northern parts – Nthongoni location with targeted villages including Matangini, Yumbuni, Mangelete, Nthongoni, Kongo and California which border the park.

The third site was Mutara ranch which is a government owned beef cattle ranch located in Laikipia County. The climate is predominantly semi-arid and the ranch is a habitat for feral baboons among other animals (Kenyampya, 2015). The baboons share the resources with the cattle and humans hence the higher risk of zoonotic parasite transmission. Humans also live around the ranch and there are tourist hotels found near the ranch. Baboons therefore move around in neighbouring farms and hotels scavenging for food hence interacting with humans and this is what necessitated its selection.

3.2 Study design

The study was conducted in two phases. The first phase was sampling of baboons and screening for helminthes and parasites. This was carried out in three study sites namely Mutara Ranch, Laikipia County, Tsavo West National Park (TWNP) and Tana River Primate National Reserve (TRPNR). The second phase was conducting a questionnaire survey in people staying near the parks two parks, TWNP and TRPNR hence interact with the baboons. Baboon studies involved trapping the animals then sampling and physical examination and realising for Tsavo and Tana River while the Laikipia baboons were relocated to Institute of Primate Research and also sampled. The samples collected
were blood, feaces and ectoparasites and the animals were also physically examined. The Laikipia baboons were later treated for helminthes and then sampled again one month post treatment to determine the haematology response associated with helminthes infection. These baboons were also used for studying pathology associated with helminthes infection. Eight baboons with highest intensities of various helminthes and two baboons negative for helminthes were selected and euthanized. The carcasses were examined for gross pathology then liver, lungs, small intestines and large intestines sampled for histopathology analysis. These tissues were stained with Hematoxylin and Eosin stain and then examined. All ages and sexes were sampled for the study from all the study sites.

The baboon studies were cross sectional studies in which they were screened at one time point.

Sampling of questionnaire respondents was multistage whereby the villages along the park borders were purposively selected then two hundred homesteads randomly selected and an adult member of the homestead interviewed.

3.3 Study subjects

In TRPNR and TWNP there are yellow baboons (*Papio cynocephalus*) while in Laikipia’s Mutara ranch we have olive baboons (*Papio anubis*). Baboons were used in the study due to their closeness physiologically to humans and their high numbers in the wild and high ease interaction with humans compared to other non-human primates.
(VandeBerg et al., 2009). Sixty olive baboons (Papio anubis) were sampled in Tana River, forty seven in Tsavo and forty in Mutara Ranch. In Tana River and Tsavo, these animals were trapped in their natural habitat, sedated, examined clinically, sampled for blood as well as ectoparasites then released. But for Mutara ranch, Laikipia County, the animals were trapped and transported to the Institute of Primate Research and used for the experiment in captivity.

3.4 Sample size determination

3.4.1 Sample size for baboons

The sample size for baboons was determined as follows.

Using the formula \( n = \frac{1.96^2 P (1-P)}{d^2} \) (Pfeiffer, 2002) Where \( n \) = sample size, 1.96 is the Z value at 95%; \( P \) = prevalence \( d \) = the precision. Prevalence for general surveys of primate parasites, it was assumed a prevalence of 5% (Leech and Sellers, 1979).

Precision was also set at 5%.

Therefore the sample size, \( n = \frac{1.96^2 \times 0.05 \times (1-0.05)/0.05^2}{1} \)

\( n = 72.99 \) animals

Hence a minimum of 73 were to be sampled for the whole study hence a minimum of 25 animals per site for the three sites. From Tsavo 47, animals were sampled, Tana River 60 and Laikipia 43 making a total of 150 baboons. This sample size was higher than the calculated because of the ease of trapping of baboons at different sites with the aim of achieving at least 25 baboons per site. As expected the baboons from different sites were easily lured into traps than from other sites hence the number varied from site
to site. The higher sample size was therefore an added advantage for better results statistically.

3.4.2 Sample size for questionnaire respondents

The formula below according to Israel (1992) was used to determine the number of respondents to interview.

\[ n = \frac{N}{1+N(e)^2} \]

Where \( n \) is the sample size, \( N \) is the population size, \( e \) is the level of precision which in this case was set at 5% (0.05).

The population of Nthongoni location which was targeted for the survey had a population of 4577 as at 2009 national census while Guano location in Tana River had a population of 1388 (Kenya National Bureau of Statistics, 2009).

Therefore the total population \( N \) was 1388+4577=5965

Hence \( n = \frac{5965}{1+5965\,(0.05)^2} \)

\[ = 5965/16 \]
\[ = 373 \text{ respondents} \]

A total of 397 respondents were interviewed (200 from Tana River and 197 from Tsavo).
3.5 Field trapping and anesthesia of baboons

Baboons were trapped in the wild with trap-door mechanism. Initially the animals were habituated to the traps by feeding them for three days around and inside the cage with open doors. Once the animals comfortably fed in the cage, the traps were set by connecting maize bait to the cage door using a thread aided by rollers. Once the baboons picked the bait, the thread would be cut and this would release the cage door hence closure (IPR, 2009). The cage door was then secured using a binding wire and the animal anaesthetized by darting using a short range blow dart. At the time of setting the traps, the anesthesia was also prepared and loaded in the blow dart so as to anesthetize the animal immediately once trapped to reduce stress as much as possible.

Xylazine hydrochloride (HCl) / Ketamine hydrochloride mixture at the ratio of 20:1 respectively at a dose rate of 10mg/kg (0.5mg/kg and of Ketamine HCl and 9.5mg/kg of Xylazine HCl) body weight was administered intramuscularly for anesthesia. Juveniles which are normally 5-10 kg were given 0.5-1ml of the anesthetic (100mg/ml concentration), female adults and sub-adult males which normally weigh between 10 – 18 kg were given a range of 1-1.8 ml while adult males which are normally between 18 kg and 30 kg were given a range of 1.8-3ml. The volume of anesthetic was decided by the veterinarian who approximated the weight of the animal from observation following Institute of Primate Research, Standard Operating Procedures (IPR, 2009). Once the animal was under deep sedation, they were carried from the cage and placed on a towel laid on a flat surface under a shade. The animal was given an identification mark by
shaving in the upper fore arm to avoid re-sampling. Data on location, species, sex, age were recorded in a book. The animal was examined clinically then sampled for blood in EDTA, fecal and ectoparasites. These procedures were carried out in accordance to the IPR SOPs, (2009). The animals were then observed for recovery from anesthesia and if this took more than 30 minutes then the anesthesia was reversed using Atipamazole hydrochloride (Atepam®, Cipla ltd, India) at 0.5 mg/kg body weight to hasten the recovery.

3.6 Physical examination

Physical exam involved observation of general body condition of the animal which was graded as very good, good, fair and poor qualitatively by a veterinarian. The body temperature was taken from the rectum using a digital thermometer. Inguinal and auxiliary lymph nodes were examined by palpation to check for enlargement and consistency. The whole skin was examined to check for any lesions, parasites and any other abnormalities. Ectoparasites that were spotted were picked and preserved in 10% formalin for identification in the laboratory. The animal identification linking age, sex, and marking plus the clinical findings were then recorded in a laboratory book.

3.7 Blood sampling

Four milliliters of blood was drawn from femoral vein into EDTA tubes. The EDTA blood was kept in a cool box immediately after collection. The blood was used for preparation of thin and thick blood smears before giemsa staining. Thin blood smears
were used for differential counts while thick blood smears were used for determination of parasitaemia under oil immersion. The remaining blood was frozen at -20\(^{0}\)C and later used for molecular characterization of hemoparasites by Polymerase Chain Reaction (PCR).

### 3.8 Fecal sampling

Fresh fecal samples were collected from the cages after trapping the animals and those that had not defecated, a sample was collected from the rectum using one finger. The fecal samples were preserved in 10% formalin and later used to perform qualitative and quantitative analysis for gastrointestinal (GIT) helminths. The quantitative analysis gave eggs per gram (EPG) counts which were also used to compare with the WHO thresholds for light, moderate or heavy infection in the animals (Table 3.1).

<table>
<thead>
<tr>
<th>Parasite</th>
<th>Light</th>
<th>Moderate</th>
<th>Heavy</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. lumbricoides</em></td>
<td>1-4,999</td>
<td>5,000-49,999</td>
<td>&gt;50,000</td>
</tr>
<tr>
<td><em>T. trichiura</em></td>
<td>1-999</td>
<td>1,000-9,999</td>
<td>&gt;10,000</td>
</tr>
<tr>
<td>Hookworms</td>
<td>1-1,999</td>
<td>2000-3999</td>
<td>&gt;4000</td>
</tr>
<tr>
<td><em>S. mansoni, S. japonicum</em></td>
<td>1-99</td>
<td>100-399</td>
<td>&gt;400</td>
</tr>
<tr>
<td><em>S. haematobium</em></td>
<td>&lt;50egg/10ml urine</td>
<td>-</td>
<td>&gt;50egg/10ml or visible haematuria</td>
</tr>
</tbody>
</table>

Table 3.1: Helminth infection intensities (EPG) according to WHO, (2002)
3.9 Questionnaire administration

Questionnaires were administered to attest the level of interaction, knowledge, attitude and behavior of the community towards non-human primates and give an insight on the risk factors for zoonosis (Appendix I). This targeted communities dwelling adjacent to the TRPNR and TWNP. One hundred and ninety seven people and ten key informants were interviewed in Guano location in Tana River and two hundred respondents plus ten key informants were interviewed in Nthongoni location in Tsavo West. In TRPNR, villages sampled were Baomo, Vukoni, Makere, Maroni, Kipendi, Hara and Wenje. Tsavo villages on the other hand were Califonia, Kathiani, Kitheini, Kongo, Mangelete, Matangini, Mitasyano, Ngiluni and Yumbuni. Simple random sampling was used for sampling households after getting the total population from the locations. The key informants included the village elders, area chiefs, public health officials and health specialists.

3.10 Laboratory analysis

The feecal samples were screened for gastro-intestinal helminths using formal ether technique as described by Munene et al. (1998). Egg per gram (EPG) counts was also carried out on the feaces as described by Gillespie (2006) for quantitative analysis (Appendix II). Thin and thick blood smears stained using Giemsa stain. The slides were examined for hematology profile and hemoparasites. Parasitaemia was also determined from the slides for all the hemoparasites and reported as percentage parasitaemia. The frozen EDTA whole blood was thawed and used for molecular characterization of
hemoparasites using PCR. Formalin fixed feecal samples were used for qualitative and qualitative analysis of gastrointestinal helminths.

Animals from Mutara ranch, Laikipia County, were trapped and transported to the Institute of Primate Research where they were used for the studies. All animals were sampled twice for stool and blood. The first was for screening feecal helminthes and a haematology profile. The animals were then treated with dewormers; Albendazole® (K.A. Malle pharmaceuticals, India) at 7.5 mg/kg body weight and Ivermectin® (Zuche pharmaceuticals Pvt Ltd, India) at 200 µg/kg body weight when found positive for any worms and a second sample of both feecal and EDTA blood was then taken one month post treatment. The feecal samples in the second sampling were used to check for the drug response while the blood sample was used to check for any changes in the hemogram.

3.11 Gross pathology and histopathology

Ten animals (eight positive and two negative for GIT helminths) were selected for helminth pathology as follows; eight animals with highest levels of infestation with helminths were purposively selected using first sample feecal analysis results and euthanized (using Euthatal®, pentobarbitone 200mg/ ml, Vector laboratories, UK) alongside two negative controls. The animals were examined for gross pathology then various tissues including liver, lungs, small intestines and large intestines collected for histopathology processing using Hematoxylin and Eosin stain. Gross pathology
examination was performed by observation of the tissues for any pathological changes and images taken. Histopathology was performed on the tissues which had observable gross pathology as well as the normal tissues from the negative control baboons. The tissues were cut in thin pieces of not more than 2 cm thick and then fixed in 10% formalin. They were allowed to fix in formalin for one month before being embedded in paraffin wax (Fisher et al., 2008) (Appendix V). After embedding, the blocks were then sectioned at a thickness of 10 µm then stained using Hematoxylin and Eosin stain (Fisher et al., 2008) (Appendix VI).

3.12 Polymerase Chain Reaction

This technique is highly sensitive and specific diagnostic tool hence can detect any parasites missed out by thin blood smears though it can also detect previous infections. Multiplex PCR was used in the procedure. DNA extraction was carried out on the whole EDTA blood using Quick-gDNA™ MiniPrep kit (Zymo Research Corporation, USA) as per the kit protocol (Appendix vii) and then stored at -20°C. The parasite-specific DNA were then amplified using specific primer sets for Hepatocystis kochi (F-5'-CATTTACACGGTAGCATAATCCTT-3' and R-5'-GGAATGTTTTTCAACATTGCAT-3'), Entopolypoides macaci (F-5'-ATACAGCGAAACTGCAATG-3', R-5'-GAAGGGTTTAGATCCCATCA), Babesia microti (F-5'-CCTGCGGCTTAATTTGACTC-3', R-5'-GGATCACTCGATCGGTAGGA-3') (Caccio et al. 2000, Tung et al, 2009). A master mix of a total of 25 µl of the PCR product was prepared in 0.2 ml thin walled PCR tube
as for each parasite using Thermo-Scientific™ (Thermo-Scientific Fisher-USA) Kit.

The master mix contained each of the above primers separately, PCR water, 10X buffer, Magnesium chloride, dNTPs, taq polymerase enzyme and the DNA sample. These were in varied amounts depending on the primer requirements as outlined in Table 3.2.

**Table 3.2: Master Mix composition for each parasite in volumes**

<table>
<thead>
<tr>
<th>Reagent (µl)</th>
<th><em>Hepatocystis kochi</em></th>
<th><em>Babesia microti</em></th>
<th><em>Entopolypoides macaci</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>PCR water</td>
<td>17.65</td>
<td>17.35</td>
<td>16.85</td>
</tr>
<tr>
<td>10 x buffer</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td>MgCl₂</td>
<td>1.25 of 50nM</td>
<td>1.5 of 25nM</td>
<td>2 of 25nM</td>
</tr>
<tr>
<td>dNTPs</td>
<td>0.5 of 10nM</td>
<td>0.5 of 20nM</td>
<td>0.5 of 10nM</td>
</tr>
<tr>
<td>Forward primer</td>
<td>1.25</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>Reverse primer</td>
<td>1.25</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>Taq polymerase</td>
<td>0.1</td>
<td>0.15</td>
<td>0.15</td>
</tr>
<tr>
<td>DNA sample</td>
<td>0.5</td>
<td>2.5</td>
<td>2.5</td>
</tr>
</tbody>
</table>

The master mix was then transferred to the thermocycler and conditions set for amplification set for each parasites as outline in Table 3.3.
Table 3.3: PCR amplification conditions for the three parasites

<table>
<thead>
<tr>
<th></th>
<th><em>Hepatocystis kochi</em></th>
<th><em>Babesia microti</em></th>
<th><em>Entopopoides macaci</em></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Initial denaturation</strong></td>
<td>95°C for 2 minutes</td>
<td>95°C for 5 minutes</td>
<td>94°C for 5 minutes</td>
</tr>
<tr>
<td><strong>Denaturation</strong></td>
<td>95°C for 45 seconds</td>
<td>95°C for 30 seconds</td>
<td>94°C for 30 seconds</td>
</tr>
<tr>
<td><strong>Primer annealing</strong></td>
<td>55°C for 30 seconds</td>
<td>59°C for 30 seconds</td>
<td>62°C for 30 seconds</td>
</tr>
<tr>
<td><strong>Extension</strong></td>
<td>68°C for 1 minute</td>
<td>72°C for 1 minute</td>
<td>72°C for 1 minute</td>
</tr>
<tr>
<td><strong>Final extension</strong></td>
<td>72°C for 5 minutes</td>
<td>72°C for 9 minutes</td>
<td>72°C for 5 minutes</td>
</tr>
</tbody>
</table>

After amplification, the PCR products were visualized on 2% agarose gel stained with ethidium bromide. This was achieved by preparing the agarose then adding 5 µl of ethidium bromide. The gel was then poured on the electrophoretic plate to set. Thermo scientific orange DNA loading dye (Inqaba – South Africa) was mixed with both the 100 base pair ladder and samples on parafilm paper at volumes of 5 µl of the sample and 5 µl of the dye. One hundred base pair ladder was placed in the first well to give the parasite genome size and the samples placed on the remaining wells. Electrophoresis was run on the gel at 100 volts for 1 hour and 30 minutes then visualized under UV light then photographed (IPR 2009).
3.13 Statistical analysis

The results for prevalence of parasites, egg counts, parasitaemia and questionnaire survey were uploaded in Windows Excel then exported to SPSS and PAST which was used for analysis. Statistical significance was set at 95% with a P value of less than 0.05 considered as significant. Point prevalence of gastrointestinal (GIT) helminths was determined for the three study sites. Kruskal-Wallis test was carried out to determine any significant difference in the GIT helminth prevalence among the three study sites. Mann-Whitney test was performed to determine if there was any significant difference in GIT helminth prevalence between pretreatment and post treatment among Laikipia baboons. One way ANOVA was also carried to determine significant difference in mean GIT helminth intensities in baboons from the three study sites. Analysis of variance was also carried out to determine significant difference in hemoparasite intensities among the parasites in each of the study sites.

Mann-Whitney test was carried out to determine if there was any significant difference in the prevalence of haemoparasites between Tana and Tsavo. Eggs per gram (EPG) counts were determined and mean and standard deviation determined using SPSS software. One way Analysis of Variance (ANOVA) was also carried out to determine if there was any significant difference in the mean leucocyte differential counts among the sites. Chi-square ($\chi^2$) test was also carried out to determine if there was any association between $H. kochi$ infection and fever and also association between $H. kochi$ infection and superficial lymph node enlargement reported for Tsavo and Tana River baboons.
Frequencies of the risk factors for zoonosis transmission were determined for each risk factor for both Tsavo and Tana River. Kruskal-Wallis was performed to determine if there was any significant difference in the risk factors between Tsavo and Tana River.

3.14 Ethical approval

The study was approved by the Ethical and Scientific Review Committee of the Institute of Primate Research review number IRC/15/11 (Appendix IV).
CHAPTER FOUR: RESULTS

4.1 Prevalence and intensity of gastrointestinal tract helminths in baboons from Tana River Primate National Reserve, Tsavo West National Park and Mutara Ranch, Laikipia

4.1.1 Prevalence of gastrointestinal tract helminths in baboons from the study sites

Out of a total of 150 baboons sampled, 60 were from Tana River, 47 from Tsavo and 43 from Laikipia. Point prevalence of gastrointestinal tract (GIT) helminths was determined and the results indicated that Tana River Primate National Reserve (TRPNR) baboons had the highest prevalence of Strongyloides (77.8%), Trichuris (27.8%) and Enterobius (14.8%) which was significantly higher than the other sites (P<0.05). Laikipia had the highest prevalence of Oesophagostomum (80.5%), and second highest Strongyloides (64.7%) and Trichuris (7.3%) and the lowest in Enterobius (1.2%). Tsavo West National Park (TWNP) baboons on the other hand had the highest prevalence in S. mansoni (2.1%) and this was the only site where the parasite was present; the site also had the second lowest prevalence in Oesophagostomum (25.5%), Enterobius (8.5%) and the lowest in Strongyloides (23.4%) (Figure 4.1a). Egg samples of various helminths observed are presented in Figure 4.1b. Overall, Laikipia had the highest of the helminth prevalence which was also significantly higher (P<0.05) than the other sites followed by Tana River which was significantly higher (P<0.05) than Tsavo that had the lowest. With regard to parasites, Oesophagostomum had the highest prevalence rates (80.5%) followed by Strongyloides (77.8%) then Trichuris (27.8%), Enterobius (14.8%) and lastly S. mansoni (1.2%).
Figure 4.1a: Prevalence of gastrointestinal tract helminths among baboons from the study sites
Figure 4.1b: Eggs of helminthes reported in baboons from the study sites. (i) *Trichuris* egg with bipolar plugs. (ii) *Oesophagostomum* egg with morula inside and ovoid shape, (iii) *Schistosoma mansoni* egg – with typical lateral spike, (iv) *Enterobius* egg flattened on one side with thick wall and a developing embryo inside, (v) *Strongyloides* egg with embryo inside

4.1.2 Prevalence of gastrointestinal helminths in baboons from Laikipia pre and post-treatment

Following treatment with Albentazole and Ivermectin, *Oesophagostomum* reduced from 80.5% to 0%, *Strongyloides* from 64.7% to 2.4% and *Trichuris* from 7.3% to 0%. *Enterobius* which had a very low prevalence of 1.2% reduced to 0%. Analysis using
paired T test indicated that there was a significant reduction in the prevalence of all the parasites post treatment (P<0.05) (Figure 4.2).

![Graph showing prevalence of helminths among Laikipia baboons pre-treatment and post-treatment](image)

**Figure 1.2: Prevalence of helminths among Laikipia baboons pre-treatment and post-treatment**

### 4.1.3 Infection intensity of helminth in baboons from the three study sites

In regard to parasite intensities, *Strongyloides* was the highest in Tana River (mean 136±82 Eggs Per Gram (EPG) of feaces) and Laikipia (mean 112±57 EPG of feaces) with Tsavo having the least (mean 105±59 EPG of feaces). There was a significant difference between the study sites (F=21.125; P<0.05). Post hoc analysis indicated that Tana River baboons had significantly higher intensity of Strongyloides than Tsavo or Laikipia (P<0.05). There was no significant difference of infection intensity between Tsavo and Laikipia baboons (P>0.05). *Oesophagostomum* was second highest with
Laikipia having the highest mean of 108±54 EPG of faeces followed by Tana River (mean of 105±40 EPG of faeces) and lastly Tsavo (mean of 99±47 EPG of faeces). There was, however, no significant difference in *Oesophagostomum* infection intensity in baboons among the sites (P>0.05). *Enterobius* was the next with Tsavo leading (88±35 EPG) followed by Tana River (mean 62±23 EPG of faeces) and finally Laikipia (mean 47±40 EPG of faeces) with a significant difference between the three sites (P>0.05). *Trichuris* was the second last in intensity with the highest mean being reported in Tana River (mean 79±25 EPG of faeces) followed by Laikipia (mean 60±27 EPG of faeces) and finally Tsavo (mean 53±35 EPG of faeces) with a significant difference between the sites (F=18.324, P<0.05). Post hoc analysis indicated that Tana River baboons had a significantly higher intensity of *Trichuris* infection than both Laikipia and Tsavo baboons (P>0.05). Laikipia baboon infection intensity was also significantly higher than Tsavo baboons (P>0.05). The lowest in intensity among the helminths was *S. mansoni* which was only reported in Tsavo (mean 50±34 EPG of faeces) (Figure 4.3).
4.2 Prevalence and infection intensity of hemoparasites in baboons from Tana River Primate Reserve, Tsavo West National Park and Mutara Ranch, Laikipia

4.2.1 Prevalence of hemoparasites in baboons from the three study sites in blood smears

Examination of blood smears slides showed that Laikipia baboons were negative for haemoparasites. In both Tana River and Tsavo, the highest prevalent parasite was *Hepatocystis kochi*. Tana River baboons had a higher prevalence of *Hepatocystis* (70%) than Tsavo (64.4%) but no *Babesia* (0%) and no *Entopolypoides* (0%) was reported in the baboons from the site. Tsavo baboons had an equal prevalence of both *Babesia* (4.4%) and *Entopolypoides* (4.4%) (Figure 4.4, 4.5a, 4.5b). There was no significant
difference in prevalence of *H. kochi* parasites between the two sites (P=0.8217).

**Figure 4.4: Prevalence of hemoparasites among baboons from Tsavo and Tana River**

**Figure 4.5: Giemsa stained blood smear with** (a) erythrocyte infected with *Entopolypoides* (top arrow) and *Hepatocystis kochi* (bottom arrow). (b) Erythrocyte infected with *Hepatocystis kochi* (top arrow) and *Babesia* (bottom arrow) (X 100)
4.2.2 *Hepatocystis kochi* parasitaemia in baboons from Tsavo and Tana River by blood smears

*Hepatocystis kochi* being the most prevalent, its parasitaemia was determined for the two study sites; Tana River and Tsavo. The mean parasitaemia was determined and baboons from Tana River had a higher mean (0.38\%±0.15\%) compared to those from Tsavo (0.27\%±0.1\%) (Figure 4.6), but there was no significant difference in parasitaemia between the two sites (P=0.0889).

![Bar chart showing mean parasitaemia of *H. kochi* in Tana River and Tsavo baboons](chart.png)

**Figure 4.6:** Mean *Hepatocystis kochi* parasitaemia in Tana River and Tsavo baboons. Data represents mean *H. kochi* parasitemia ± Standard deviation.

4.2.3 Prevalence of hemoparasites by PCR in Tana River and Tsavo baboons

Figure 4.7 is sample gel for PCR products of hemoparasites identified in the study sites.
Figure 4.7: Representative gel showing the polymerase chain reaction product. Lane 1 and 17 were 100bp molecular ladders. Lane 2-10 for *H. kochi* (250bp) with lane 7 negative and lane 11-16 *Entopolypoides macaci* (435bp) all being negative except lane 14. After amplification which was performed as indicated in table 4.7, the PCR products were visualized on agarose gel stained with ethidium bromide. Two percent agarose was prepared then 5 µl of ethidium bromide added and the gel poured on the electrophoretic plate to set. Thermo scientific orange DNA loading dye (Inqaba – South Africa) was mixed with both the 100 base pair ladder and samples on parafilm paper at volumes of 5 µl of the sample and 5 µl of the dye. One hundred base pair ladder was placed in the first well to give the parasite genome size and the samples placed on the remaining wells. Electrophoresis was run on the gel at 100 volts for 1 hour and 30 minutes then visualized under UV light then photographed.

Eighty seven percent (40/46) of Tsavo animals were positive for *Hepatocystis kochi* using PCR, 10.8% (5/46) for *Babesia microti* and 8.7% (4/46) were positive for *Entopolypoides*. Ninety percent (90%) (54/60) of Tana animals were positive for *Hepatocystis*, 16.7% (10/60) for *Babesia microti* and 5% (3/60) for *Entopolypoides* (Figures 4.8).
4.2.4 Comparison between hemoparasites prevalence by PCR and blood smear in Tana River and Tsavo baboons

PCR was more sensitive than blood smear as it detected higher prevalences of the haemoparasites. PCR detected *Hepatocystis kochi* at 90% as opposed to 64.4% detected by blood smears in Tsavo and 87% as compared to 70% detected by blood smears in Tana River. It also detected higher prevalences of *Babesia* in Tsavo (10.8% compared to 4.4% in blood smears) and *Entopolypoides* as well in Tsavo (8.7% compared to 4.4% in blood smears). The test was able to detect *Babesia* and *Entopolypoides* in Tana River baboons which were absent in blood smears (Figure 4.8).
4.2.5 Ectoparasites and clinical outcomes of baboons from Tsavo and Tana River

In both sites, the baboons were infested with hard (Ixodidae) ticks with Tsavo baboons having a higher prevalence (28%) of ticks than TRPR (23.3%) though the difference was not significant (P>0.05). The species of ticks found in the baboons were *Rhipicepalus pulchellus*, *Rhipicephalus simus simus* and *Hyalomma truncatum* (Figure 4.9).

![Figure 4.9: Ticks recovered from the baboons in Tana River and Tsavo preserved in formalin (X40). a) Rhipicepalus pulchellus male visible colouration (b) Boophilus sp engorged female, (c) Rhipicephalus simus simus female (d) Hyalomma truncatum ventral view with prominent anal groove and mouth parts (capitulum) and (e) Hyalomma truncatum dorsal view with prominent scutum and capitulum.](image)

The location of ticks varied from armpit, inguinal, rectal regions and ventral abdomen. There were also a higher proportion of baboons with erythematous lesions (55% from Tsavo and 48.3% from Tana) suggesting that the ticks had recently fallen off. The
difference was also not significant between the sites (P>0.05). All Tana River baboons had fever (100%) of between 38.8°C and 41.6°C and with an average of 40°C while in Tsavo, 98% had fever of between 38.6°C and 41.8°C with an average of 40.3°C as opposed normal body temperature of between 37.5-38°C. There was no significant difference in the fever between the sites (P>0.05). Examination of superficial lymph nodes; inguinal and axillary lymph nodes indicated enlargement of sizes to between 0.8 cm and 1.2 cm with an average of 1cm which was more than twice the normal size of 0.4 cm in adults. In Tsavo, all the baboons (100%) had lymph node enlargement which was significantly higher (P<0.05) than Tana baboons (67%) (Figure 4.10).

**Figure 4.10: Prevalence of clinical signs and ticks in Tana River and Tsavo baboons.** Erythema lesions appeared in areas where ticks are normally lodged, fever range was between 38.6°C-41.8°C, superficial lymph nodes were enlarged twice or over the normal size

A Chi square test indicated that there was an association between *H. kochi* infection and lymph node enlargement (Tsavo: $\chi^2=4.893$, df=1, P=0.026; Tana: $\chi^2=8.100$, df=1,
P=0.004). *Hepatocystis kochi* infection was also associated with fever in baboons from both sites (Tsavo: $\chi^2=35.103$, df=1, P=0.001; Tana: $\chi^2=3.920$, df=1, P=0.045).

### 4.3 Leukocyte response to helminth infections

#### 4.3.1 Leukocyte differential counts in baboons from the three study sites

Mean leukocyte differentials, when compared to the normal ranges of white blood cell (WBC) counts were within the normal range (3900-16000/µl of blood) for all the sites. Tsavo mean counts were 4900/µl, Tana mean counts were 15000/µl while Laikipia mean counts were 9600/µl. Analysis of mean differential leucocyte counts between sites was conducted and there was a significant difference in the white blood cell (WBC) counts (P<0.05) with Tana having the highest (15000±3000/µl), followed by Laikipia (9600±2300/µl) and lastly Tsavo (4900±1700/ µl). Generally, Tana River baboons had the highest differential leucocyte counts followed by Laikipia and lastly Tsavo baboons. Neutrophil counts were within the normal range (2000-5000/µl) for Tsavo baboons (mean counts of 1700/µl) but there was a slight neutrophilia in both Laikipia (mean count of 5400/µl) and Tana (mean count of 6000/µl). There was a significant difference in neutrophil counts (P<0.05) between the sites with Tana also having the highest (6000±1400/µl) followed by Laikipia (5400±1800/ µl) and lastly Tsavo (1700±500/µl). Lymphocytosis was reported in baboons from Tana (mean counts of 8000/µl) as opposed to normal range of 2000-5600/µl. Laikipia (mean counts of 3700/µl) and Tsavo (mean counts of 2500/µl) lymphocyte counts were within the normal ranges.
There was a significant difference in the lymphocyte counts between the sites with Tana still having the highest (8000±2500/µl) followed by Laikipia (3700±1300/µl) and finally Tsavo (2500±600/µl). Eosinophil counts were within the normal ranges (0-5000/µl) in all the sites (Tana mean counts were 400/µl, Tsavo mean counts were 130/µl and Laikipia mean counts were 290/µl). However, there was no significant difference in eosinophil, monocyte and basophil counts (P>0.05). The monocyte counts were also within the normal ranges (0-1000/µl) (Tsavo mean counts were 110/µl, Tana, mean counts were 300/µl and Laikipia mean counts were 320/µl). Equally basophil counts were within the normal ranges (0-100/µl) (Tsavo mean counts were 90/µl, Tana mean counts were 140/µl and Laikipia mean counts were 130/µl (Table 4.1).

Table 4.1: Mean leucocyte counts ± standard deviation for the three study sites and the P values for significance among the baboons from the sites

<table>
<thead>
<tr>
<th>Parameter</th>
<th>WBC x 10^3/µl</th>
<th>Neutrophils x10^3/µl</th>
<th>Lymphocytes x 10^3/µl</th>
<th>Eosinophils x 10^3/µl</th>
<th>Monocytes x 10^3/µl</th>
<th>Basophils x 10^3/µl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal range</td>
<td>3.9 - 16</td>
<td>2- 5.2</td>
<td>2-- 5.6</td>
<td>0-5</td>
<td>0-1</td>
<td>0-1</td>
</tr>
<tr>
<td>Tsavo</td>
<td>4.9±1.7</td>
<td>1.7±0.5</td>
<td>2.5±0.6</td>
<td>0.13±0.08</td>
<td>0.11±0.04</td>
<td>0.09±0.04</td>
</tr>
<tr>
<td>Tana</td>
<td>15±3</td>
<td>6±1.4</td>
<td>8±2.5</td>
<td>0.4±0.2</td>
<td>0.3±0.2</td>
<td>0.14±0.07</td>
</tr>
<tr>
<td>Laikipia</td>
<td>9.6±2.3</td>
<td>5.4±1.8</td>
<td>3.7±1.3</td>
<td>0.29±0.1</td>
<td>0.32±0.13</td>
<td>0.13±0.03</td>
</tr>
<tr>
<td>P value</td>
<td>0.000</td>
<td>0.005</td>
<td>0.001</td>
<td>0.875</td>
<td>0.654</td>
<td>0.974</td>
</tr>
</tbody>
</table>

Normal values (Foy et al., 1965)
4.3.2 Hematological parameters in Laikipia baboons pre and post treatment against gastrointestinal helminths

Hematology parameters for Laikipia baboons were compared pre and post treatment against helminth. There was a decrease in WBC mean counts from 11400/µl to 9800/µl of blood though this was within the normal ranges. The leucocyte differentials were all within the normal ranges except neutrophil counts which were slightly higher pretreatment (5700/µl while maximum normal limit is 5200/µl). Neutrophil counts also reduced marginally from 5700/µl to 5100/µl post treatment. There was also a reduction of lymphocyte counts post treatment from 4300/µl to 4100/µl. Monocytes increased slightly post treatment from 400/µl to 450/µl whereas eosinophils reduced from 400/µl before treatment to 300 post-treatment. Basophils also reduced marginally from 100/µl to 90µl post treatment. Following treatment, there was no significant difference in means of all leucocytes using T-test (P>0.05, Table 4.2).

Erythrocyte parameters had significant changes following treatment (P<0.05) except mean corpuscular volume (MCV) (P>0.05) with all of the parameters having increased. The mean red blood cell (RBC) counts increased from 5.1 x 10⁶/µl pre-treatment to 5.3 x 10⁶/µl post treatment. Mean hemoglobin (HB) amount equally increased following treatment from 12g/l to 13.3g/l. Packed cell volume (PCV) mean levels increased from 40.1 l/l pre-treatment to 42.1 l/l post treatment while MCV mean increased slightly from 78.6 fl/cell pre-treatment to 78.7 fl/cell post-treatment. Mean corpuscular hemoglobin (MCH) mean also increased from 24.5 pg/cell pre-treatment to 24.9 pg/cell post-
treatment while mean corpuscular hemoglobin concentration (MCHC) mean increased from 31.2g/cell pre-treatment and 31.6g/cell post-treatment. There was no significant change in platelet counts (P>0.05) with a marginal increase from 250.3 x 10^3/µl pretreatment to 257x103/µl post treatment (Table 4.2).

Table 4.2: Mean hematology parameters of Laikipia baboons pre and post treatment against helminths and P values from t test

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normal range</th>
<th>Pre-treatment</th>
<th>Post-treatment</th>
<th>t-value</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC x 10^3</td>
<td>3.9-16</td>
<td>11.4</td>
<td>9.8</td>
<td>1.946</td>
<td>0.059</td>
</tr>
<tr>
<td>Neutrophils x 10^3</td>
<td>2.5.2</td>
<td>5.7</td>
<td>5.1</td>
<td>1.092</td>
<td>0.282</td>
</tr>
<tr>
<td>Lymphocytes x 10^3</td>
<td>2-5.6</td>
<td>4.3</td>
<td>4.1</td>
<td>0.586</td>
<td>0.561</td>
</tr>
<tr>
<td>Monocytes x 10^3</td>
<td>0-1</td>
<td>0.4</td>
<td>0.45</td>
<td>-1.870</td>
<td>0.069</td>
</tr>
<tr>
<td>Eosinophils x 10^3</td>
<td>0-5</td>
<td>0.4</td>
<td>0.3</td>
<td>1.720</td>
<td>0.093</td>
</tr>
<tr>
<td>Basophils x 10^3</td>
<td>0-1</td>
<td>0.1</td>
<td>0.09</td>
<td>1.688</td>
<td>0.098</td>
</tr>
<tr>
<td>RBC x 10^6/µl</td>
<td>4.6-5.3</td>
<td>5.1</td>
<td>5.4</td>
<td>-3.571</td>
<td>0.001</td>
</tr>
<tr>
<td>HB (g/dl)</td>
<td>11.7-14.5</td>
<td>11.9</td>
<td>13.3</td>
<td>-2.241</td>
<td>0.031</td>
</tr>
<tr>
<td>PCV (%)</td>
<td>36-41</td>
<td>40.2</td>
<td>42.1</td>
<td>-3.642</td>
<td>0.001</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>74-80</td>
<td>78.6</td>
<td>78.7</td>
<td>-0.108</td>
<td>0.914</td>
</tr>
<tr>
<td>MCHC (p/d)</td>
<td>30.9-33.9</td>
<td>31.2</td>
<td>31.6</td>
<td>-5.972</td>
<td>0.001</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>23.4-28.3</td>
<td>24.5</td>
<td>24.9</td>
<td>-4.729</td>
<td>0.001</td>
</tr>
<tr>
<td>Platelets x 10^3/µl</td>
<td>233-399</td>
<td>250.3</td>
<td>257.7</td>
<td>-0.266</td>
<td>0.792</td>
</tr>
</tbody>
</table>
4.4 Pathology associated with gastrointestinal helminthes in Laikipia baboons

The animal infected with *Oesophagostomum* grossly had nodular lesions and congestion in the large intestinal mucosa (Figure 4.11a) when compared to the normal (Figure 4.11b). Histologically, there was severe degeneration of the mucosa (Figure 4.12a) as opposed to the normal intestine that had clear secretory epithelium (Figure 4.12b). The baboon with *Strongyloides* infection mainly had lesions in the lungs which were noted histologically as fibrosis around the vessels (Figure 4.13a) compared to normal lung histology (Figure 4.13b) which had well defined alveoli. Grossly, the main pathology noted in the small intestines was intussusception (Figure 4.14a) which was associated with *Trichuris* infection as opposed to small intestine from a non-infected baboon (Figure 4.14b) which had no such pathology. Histologically, the affected area had degeneration with prominent infiltration of inflammatory cells especially the macrophages (Figure 4.15a) as opposed to the negative control (Figure 4.15b) which had a well-defined epithelium with clear secretory epithelium. The baboon with *S. mansoni* infection had lesions in the liver with periportal fibrosis on gross section (Figure 4.16a) unlike the liver from non-infected baboon with no fibrosis (Figure 4.16b). Histologically, there were areas of granuloma formation (Figure 4.17a), periportal fibrosis (Figure 4.17b) and parenchymal fibrosis (Figure 4.17c) unlike the liver from uninfected baboon which showed clear hepatocytes with no visible pathology (Figure 4.17d).
Figure 4.11: Picture of baboon large intestines. (a) baboon large intestine infested with *Oesophagostomum*. Arrow show nodular lesions which are usually caused by larva which penetrated the mucosa to develop. (b) normal mucosa

Figure 4.12: Baboon large intestine tissue stained with Hematoxylin and Eosin. (a) A section through the nodule from the animal that was infected with *Oesophagostomum* which indicates necrotic epithelium-destroyed secretory epithelium with no clear mucosa nor cells (top arrow) the sub mucosa occupied by the larval stage of the worm (bottom arrow) (X25). (b) Normal histology with clearly visible secretory epithelium, a clear sub mucosa and serosal layer (X25)
Figure 4.13: Baboon lung tissue stained with Hematoxylin and Eosin. (a) perivascular fibrosis seen in an animal which had the highest infection of *Strongyloides* x10. Due to the fibrosis there is pressure exerted on the surrounding alveoli hence collapse. (b) Normal lung with clearly defined alveoli and blood vessel x25

Figure 4.14: Picture of baboon small intestines (a) intussusception (b) normal. a) intussusception of the small intestines indicated by the arrow from a baboon infected with *Trichuris*. (b) Normal appearance from serosal surface
**Figure 4.15:** Baboon small intestine tissue stained with Hematoxylin and Eosin with intussusception from baboon infected with *Trichuris*. (a) Small intestine with degeneration of the mucosa with unclear secretory epithelium and predominance of inflammatory cells especially macrophages (pink in colour). The animal had the highest *Trichuris* infection and this section is from intussuscepted area. (b) Normal histology with typical villi and microvilli forming the epithelium, sub mucosa and serosa (x10)

**Figure 4.16:** Picture of gross liver section of baboon (a) Infected with *S. mansoni*: Liver with pathology degenerative section brownish in colour of parenchyma with thickened portal vessels indicated by the arrow. (b) Normal liver bright red with non thickened portal vessels
Figure 4.17: Baboon liver tissue (a) Granuloma formation with inflammatory cells walling off an area with *S. mansoni* egg x25. (b) Severe periportal fibrosis still in animal with *S. mansoni* eggs x 25. (c) Parenchymal fibrosis and degeneration of hepatocytes with severe congestion from an animal with *S. mansoni* eggs x 10 (d) Normal liver parenchyma with clearly visible hepatocytes and sinusoids (arrow) x 25
4.5 Risk factors associated with transmission of zoonosis at the baboon-human interface in Tsavo and Tana

4.5.1 Distribution of respondents among the sampled villages

Two hundred villagers from 35 villages and 20 key informants were interviewed in Tsavo’s Nthongoni location of Kitui County. One hundred and ninety seven respondents and 20 key informants were interviewed in Tana River’s Ndera and Guano locations which border the Tana River Primate National Reserve. In Guano location, 6 villages were surveyed and 1 in Ndera (Table 4.3).

Table 4.3: Distribution of respondents among the sampled villages

<table>
<thead>
<tr>
<th>Location</th>
<th>Village</th>
<th>No. of respondents</th>
<th>Location</th>
<th>Village</th>
<th>No. of respondent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nthongoni</td>
<td>California</td>
<td>8</td>
<td>Guano</td>
<td>Makere</td>
<td>21</td>
</tr>
<tr>
<td>Nthongoni</td>
<td>Kathiani</td>
<td>15</td>
<td>Guano</td>
<td>Maroni</td>
<td>30</td>
</tr>
<tr>
<td>Nthongoni</td>
<td>Kitheini</td>
<td>13</td>
<td>Guano</td>
<td>Kipendi</td>
<td>22</td>
</tr>
<tr>
<td>Nthongoni</td>
<td>Kongo</td>
<td>26</td>
<td>Guano</td>
<td>Wenje</td>
<td>44</td>
</tr>
<tr>
<td>Nthongoni</td>
<td>Mangelete</td>
<td>56</td>
<td>Guano</td>
<td>Vukoni</td>
<td>39</td>
</tr>
<tr>
<td>Nthongoni</td>
<td>Matangini</td>
<td>7</td>
<td>Guano</td>
<td>Hara</td>
<td>10</td>
</tr>
<tr>
<td>Nthongoni</td>
<td>Mitamaiyu</td>
<td>8</td>
<td>Ndera</td>
<td>Baomo</td>
<td>31</td>
</tr>
<tr>
<td>Nthongoni</td>
<td>Mбуkoni</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nthongoni</td>
<td>Mitasyano</td>
<td>10</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Nthongoni</td>
<td>Ngiluni</td>
<td>9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nthongoni</td>
<td>Yumbuni</td>
<td>14</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nthongoni</td>
<td>Others (&lt;5/village)</td>
<td>29</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>200</td>
<td>Total</td>
<td>197</td>
<td></td>
</tr>
</tbody>
</table>
4.5.2 Demographic characteristics of the respondents from Tsavo and Tana River

4.5.2.1 Gender of the respondents from the two study sites

Out of the 197 respondents in Tana River, 101 (51%) were males, while 96 (49%) were females whereas 88 (44%) of the 200 respondents in Tsavo were males, while 112 (56%) were females (Figure 4.18).

![Figure 4.18: Distribution of gender of the respondents from the study sites](image)

4.5.2.2 Land ownership among the respondents from the study sites

Land ownership in Tana River is mainly by inheritance (95%) with only 2.5% having bought, 1.5% leasing and 1% being squatters. Tsavo’s most inhabitants had bought land (59%) and only 33% having inherited with 3% leasing while 5% from other sources being either squatters, grabbing or being allocated by the government (relocation).
There was significant difference between the sites with regard to those that inherited land \((P=0.0001)\), and those who bought \((P=0.0001)\). There was no significant difference with regard to those who leased land \((P=0.732)\) and those who acquired land with other means such as grabbing \((P=0.0678)\).

**Figure 4.19: Land ownership in the two study sites**

### 4.5.2.3 Education levels of the respondents in the two study sites

Majority of the residents in Tana River had low education level with 13\% having no education, 58\% having primary school education; 23.4\% having secondary school level and 4.6\% having tertiary education. In Tsavo, the frequency of respondents with no education was lower (5\%) which was significantly lower than Tana residents (13\%).
Those having up to primary level being 50% in Tsavo which was not significantly different from Tana residents (58%) (P=0.0945) while those with secondary school level education being higher in Tsavo (31%) as compared to Tana (22.8%) though the difference was not significant (P=0.0866). Tsavo residents had a significantly higher frequency of individuals with tertiary education (12%) than Tana residents (4.6%) (P=0.009) (Figure 4.20).

![Education level](image)

**Figure 4.20: Education levels of respondents**

### 4.5.2.4 Land size ownership among the respondents

Most people in both Tana River and Tsavo owned land size of between 1-5 acres (62%, 56% respectively) with 27% having more than 5 acres while 11% had less than 1 acre in
Tsavo while 15% had less than 1 acre and 29% having more than 5 acres in Tana (Figure 4.21). There was no significant difference in the land sizes between the two sites (Less than 1 acre, $P=0.1146$; 1-5 acres, 0.3092, more than 5 acres, 0.9995).

![Bar chart showing land size by residents in Tsavo and Tana](image)

**Figure 4.21: Land size by residents**

### 4.5.2.5 Monthly income among the respondents from the two study sites

Most of the people had income of less than 35 USD per month in both sites (58% in Tsavo and 55.1% in Tana) with only 9.5% having earning of between 35-70 USD, 3% having 71-115USD and 6.5% earning more than 115 USD. Tana on the other hand had a slightly higher number of people who earned between 35 and 70 USD (30.3%) and 10.1% earned between 71-115 USD and only 4.5% earned more than 115 USD (Figure...
There was no significant difference between the two sites with regard to those who earned less than 35 USD and those who earned more than 115 USD (P=0.483). On the other hand there was a significant difference in those who earned between 35 USD and 75 USD (P=0.0001) as well as those who earned between 71 and 115 USD (P=0.018).

Figure 4.22: Monthly earnings of the two study sites

4.5.2.6 Occupations among the respondents in the two study sites

The main occupation for the people was farming in both sites with Tana River having slightly higher (88.3%) while Tsavo had 75% farmers. Formal employment was slightly higher in Tsavo (16%) as opposed to 7.1% in Tana River. Other occupations included
taxi and other businesses. There was significant difference in the frequency of farmers with Tana having more (P=0.0021) as well as formal occupation (P=0.0309) but with Tsavo have more. There was no significant difference in respondents who were involved in occupations other than farming between the study sites (P=0.8286) (Figure 4.23).

**Figure 4.23: Occupation of the people**

### 4.5.2.7 Types of farming practised by the inhabitants from the study sites

Inhabitants of Tana River mainly practiced crop cultivation (51.3%) which was significantly higher than those who practiced the same in Tsavo (23%) (P=0.0001). Most inhabitants in Tsavo practiced mixed farming (75%) which was also significantly higher than those who practiced the same in Tana (41.1%) (P=0.0001). There was no
significant difference between the sites with regard to those who practiced livestock farming only (Tsavo, 1.5% and Tana, 5.6%) (P=0.0547) (Figure 4.24).

![Farming type](image)

**Figure 4.24: Type of farming practiced by the respondents in the study sites**

Farming as an activity was mainly carried out by both husband and wife (68.5%). There were cases where only the husband was responsible (13.2%) or the wife alone (15.7%) with very few instances (2.5%) involving the whole family. In Tana River, crops cultivated included maize, banana, mango, green grams, sorghum, cowpeas, cassava, millet and water melon with maize and banana having the highest frequency of 67.5% followed by mangos and green grams each at 42.7% while the others were grown by 15.7% of the farmers. In Tsavo on the other hand, the main type of crops farmed included maize (94.5%), beans (74.5%) and cowpeas (42%). Most of the crops were farmed at subsistence level (56.5%), although a mixture of commercial and subsistence
farming (42.5%) was also observed. The inhabitants mainly did their farming manually (95%). There was food insufficiency in 72.5% of the respondents.

Tana River farmers kept various livestock including cattle (53.3%) only, sheep and goats (2.5%) only and chicken (31%) only. Other farmers combined several different livestock types which included cattle, sheep and goats (3%), chicken, sheep and goats (6.1%), while a small number (4.1%) kept all the animals. The animals were mostly kept by free range system (52.3%) with 46.2% being reared by both tethering and free range and only 1.5% of the respondents keeping their animals exclusively in the compounds. In Tsavo, the types of livestock kept by farmers included cattle (50%), sheep (17.5%), goats (79.5%) chicken (15%), donkeys (2.5%) and pigs (0.5%). The farmers kept the animals under confinement (45.5%), free range (14.5%) or both conditions (20.7%).

4.5.3 Risk factors associated with zoonoses transmission in Tana River and Tsavo

4.5.3.1 Food insufficiency among the respondents in the study sites

Food insufficiency was considered a risk factor because it lead to poverty which occasioned inhabitants to feed on left overs from crop raids and livestock predation. There were a very high percentage of inhabitants in both sites who attested to the fact that the food they farmed was insufficient (71.5% in Tsavo and 80.7% in Tana) with no
significant difference between the sites (P=0.071, Figure 4.25). The inhabitants had therefore to look for other sources of food which included government rations, Non – Governmental Organization (NGOs) donations and help from relatives.

**Figure 4.25: Food insufficiency among inhabitants from the study sites**

### 4.5.3.2 Sharing river water source with baboons among the respondents

There were various sources of water for both sites which ranged from tap water, borehole, well, and river and these varied between the sites. In Tsavo, there were mainly seasonal streams so people mainly relied on borehole water (52%) while others used well water (31.5%) and small percentage of 10% and 6% used tap water and river water respectively. Tana River was more advantaged in water supply since they had a permanent river flowing (River Tana) so a big percentage (76%) relied on the river
followed by bore hole water (21%) and a small amount of 3.5% relied on well water and only 0.5% could access tap water (Figure 4.26). There was a significant difference in all water sources with the P values being the same (P=0.0001) with Tsavo having higher frequencies except river/stream use where Tana was higher.

**Figure 4.26: Water sources for inhabitants from the study sites.** Tana River inhabitants mainly depended on River Tana whose banks were the habitat of the non-human primates while Tsavo inhabitants mainly sourced their water from boreholes and wells with no permanent rivers

4.5.3.3 Crop raid by baboons in the respondents farms in the study sites

Crop raid by NHPs was highest in Tana River (93.9%) yet it was still high in Tsavo too (88.5%) with no significant difference between the two sites (P=0.0741, Figure 4.27). The baboon was the most notorious in both sites with Tana River having slightly higher
frequency of 75.1% than Tsavo (65%) though not significant (P=0.268). In the same site the second most notorious was sykes monkey (34%) which was significantly higher than Tsavo (14%) (P=0.0010). Mangabeys were only reported in Tana River with a low frequency (16.7%). The vervet monkey was more significantly problematic in Tsavo (47%) as opposed to Tana River (15.7%) (P=0.0001). The colobus was also significantly higher in raids in Tana (5.5%) as compared to Tsavo (0.5%) (P=0.001) (Figure 4.28). When baboon was compared with other non-human primates, it was found to be significantly higher in crop raid (P=0.0001).

![Crop raiding in Tana River and Tsavo](image)

**Figure 4.27: Crop raiding in Tana River and Tsavo.** There was no significant difference in the crop raids (P<0.05)
Figure 4.28: Non-human Primates involved in crop raids. Baboons were significantly sited as being involved in crop raid more than the other non-human primates (P<0.05)

4.5.3.4 Livestock predation in the homesteads of the respondents from the study sites

There was a significantly high level of livestock predation in Tsavo (79.5%) as compared to Tana River (44.2%) (P=0.0001, Figure 4.33). The most notorious in livestock predation was the baboon in both sites with Tsavo being slightly higher (92.9%) compared to Tana (91.8%) although the difference was not significant between the sites (P=0.8734, Figure 4.29). However, compared to other primates, the baboons were significantly higher in livestock predation (P=0.000). This was followed by the vervet monkey (4.5%) then sykes and other wildlife both having a frequency of 1.3%.
In Tana, the vervet, sykes monkey and other animals had the same frequency of 2.7% (Figure 4.30). Overall, there was no significant difference in the other wildlife predators between the sites (P=0.7648).

**Figure 4.29: General livestock predation in the study sites.** There was a significant difference in livestock predation between the sites with Tsavo being higher than Tana (P<0.05)
Figure 4.30: Animals implicated in livestock predation. Baboons were significantly (P=0.001) higher in livestock predation compared to other non-human primates

4.5.3.5 Consumption of left overs from crop raid/predation by the respondents

Consumption of left overs from crop raid and livestock predation was a major issue in Tsavo where 66% respondents attested to the act and very low in Tana River (19.8%), which were significantly different (P=0.0001, Figure 4.31).
Figure 4.31: Consumption of leftovers from crop raid/livestock predation in the study sites. The left over consumption was significantly higher in Tsavo compared to Tana River (P<0.05)

4.5.3.6 Monkey meat consumption by the respondents from the study sites

Monkey meat consumption was low in both sites with Tsavo having a frequency of 11.5% and Tana 10.6% with no significant difference in these frequencies (P=0.8725, Figure 4.32). The reasons for consumption ranged from hunger, curiosity, revenge following raids and predation and cultural as some meat from sykes was believed to be medicinal. The most preferred meat was baboon meat in Tsavo (12%) where as in Tana it was the sykes monkey (7.5%) then baboon (2%) and vervet monkey (1%). In Tsavo the second preferred NHP was the vervet (5.5%) then the sykes (3.5%) (Figure 4.33). There was a significant difference in preferred NHP meat in all the sites (P=0.0001 for all NHPs) with Tsavo having higher frequency for baboon and vervet while Tana had higher frequency for sykes.
Figure 4.32: Monkey meat consumption in the study sites. There was no significant difference in monkey meat consumption between the two study sites (P>0.05).

Figure 4.33: Preferred Non-Human Primate meat in the two study sites. Baboon meat was the most preferred in Tsavo whereas sykes monkey meat was the most preferred among Tana River residents with all the primate species consumption being significantly different between the sites (P<0.05).
4.5.3.7 Lack of knowledge on zoonoses by the respondents from the study sites

There was a high level of ignorance on zoonosis with 81% in Tsavo and 92.4% in Tana River being ignorant about zoonotic infections and the two sites having a significant difference in the frequencies (P=0.0016, Figure 4.34).

![Graph showing comparison between Tsavo and Tana](image)

**Figure 4.34: Lack of knowledge on zoonoses.** Ignorance on zoonoses knowledge was significantly higher in Tana compared to Tsavo (P=0.0016)

4.5.4 Qualitative data from the respondents and the key informants from the study sites

During questionnaire administration, some answers demanded further probing. The remarks from the key informants (20) and respondents were sorted and categorized into different themes according to the issues they raised on the concepts addressed.
4.5.4.1 Control of monkey raids among the respondents

Most farmers were concerned over what could be done to restrict the animals in the park. Twenty eight percent (28%) of the respondents recommended an electric fence be erected to prevent the animals from straying into their farms or homesteads. They felt that the animals should be trapped and translocated. One farmer was particularly bitter with how monkeys destroy her crops, eat chicken and kids. “You should stop them from coming to our homes otherwise we will revenge” were her remarks.

Thirty six percent (36%) of the farmers however felt that an electric fence might not be an effective solution. “Monkeys are clever to cross even an electric fence”. The monkeys are also seemingly aware that the farmers cannot chase them beyond the fence, hence they don’t run very far once they cross the fence. They follow the farmers back into the farms once they retreat. Elephants were also claimed to destroy the electric fence by felling huge trees on it. This destruction combined with the fact that some areas have not been fenced allows antelopes, monkeys and elephants to continue destroying food crops. “These animals are notorious for destroying pawpaws, pumpkins and other crops. Sometimes we have to take guard the whole night”, remarked the respondent.

4.5.4.2 Lack of compensation by Kenya Wildlife Service

"We are severely punished for hunting wild animals, yet nothing is done when they destroy our crops” said a farmer. The respondents claimed that Kenya wildlife Service
(KWS) do not do anything when incidents of crop raids are reported. They felt that the poverty conditions in the area are as a result of crop raids and predation. Baboons and vervets were blamed for their tricks in stealing food from houses and baboons in particular for their strength in destroying granaries and chicken houses.

The farmers recommended for compensation for destroyed food crops and killed livestock. They also felt that KWS should compensate them for the time spent guarding their farms from wild animals or for the money spent on hired guards. They claimed that people kill monkeys as a revenge for the raids.

4.5.4.3 Non-human primates were alleged source of poverty in the study areas

“Please get rid of these monkeys. They are the main cause of poverty in this area”. These were words of one of the respondents. He further reiterated that he stopped growing tomatoes and maize due to the destruction. Other respondents gave similar remarks, some excerpts from the respondents are listed:

i. Monkeys prey on goats and chicken, and uproot maize seedlings

ii. Baboons are very destructive; "heri ndovu kuliko nyani". (Better elephants than baboons)

iii. Baboons pluck out goat's eyes and eat their kids. They also eat chicken and raid on our fruits.
iv. Monkey raids make food scarce and eventually very expensive.

v. Monkeys destroy stores, steal food from the kitchen and prey on goat kids and chicken.

vi. We have to keep day and night guard of our crops since planting to harvesting. "I stopped rearing goats due to predation" said one farmer.

vii. The land here is very fertile but farming is impossible due to destructive animals. Even chicken we cannot rear.

viii. Elephants bypass the fence and uproot mango and pawpaw trees, and there is no compensation for all this destruction.

ix. We have to keep watch over our maize until it’s harvested. Baboons are notorious in attacking goats and chicken. They cause a lot of destruction including breaking into houses.

Some respondents wondered why they are not permitted to feed on bush meat after the animals have destroyed their food crops. They felt that people have become very poor in the area due to these raids. “We waste a lot of time guarding the garden. No other work can be done”. It was reported too that some farmers have resulted to charcoal burning and sale of poles and posts from the forest when their crops are destroyed. Crops destruction, poverty and consequently hunger were blamed for bush meat hunting as well as consumption of animals that died of unknown causes. Two respondents confessed eating dead livestock without experiencing any adverse effect.
4.5.4.4 Retaliation attacks on wildlife

“We will keep poisoning them if they are not controlled”. Revenge on the animals was an issue that kept coming up in the course of this study. Farmers were categorical that they would continue killing monkeys and other wild animals if nothing is done. They admitted using farm chemicals such as ‘Furadan’- carbofuran (a carbamate pesticide) which is banned, to poison the monkeys. One farmer observed that the animals are more destructive during the dry season when even finding food for the family is a big problem. “We kill those that come to raid at such times.” Another farmer impressively gave an account of how and why they hunt elephants. She concluded by saying; “Elephants are very destructive, but then we hunt them and enjoy their meat.”

4.5.4.5 Effects of crop raids/ livestock predation on education

Children have to take care of the ‘shambas’ during the day while the parents do it over the night. The farmers observe that they may never harvest anything if this is not done. As a result, many school going children miss classes until foods are harvested. “Baboons are the reason I don’t raise any livestock or chicken. I have no children hence no one to protect the animals from baboons.”

4.5.4.6 Consumption of bush meat

Bush meat provoked mixed reactions. Some respondents said it is dangerous while others claimed it is much tastier than livestock meat. Some appeared to understand the dangers lurking in bush meat. One of the respondent said that wild animals harbor
diseases since they are never vaccinated. Some respondents also remarked that uninspected meat is dangerous, and that people have fallen sick before, from eating bush meat. Two of the participants reported that those falling ill are hesitant to report it for fear of being arrested. However, one of the key informants, a clinical officer said she had witnessed several cases of diarrhea, stomach ache and headaches resulting from bush meat consumption. Village elders and dispensaries were said to notify the veterinary office whenever such a problem was identified. Monkey meat was said to constitute bush meat in the region but none of the respondents confessed to have personally eaten monkey meat. They, however, admitted that they could have eaten it unknowingly, from poachers who hawk bush meat in the region. “Strange meat is hawked around at night. The meat is a mixture of different wild animals and it’s difficult to tell which is which”. Other respondents said that they have heard of people eating monkeys but have never seen them.

4.5.4.7 Cultural beliefs

Cultural beliefs and values influence the way people live and do things. It was reported that some farmers have a belief that a baboon arm, if chopped, dried and used as a tool in planting, could increase the crop yields. This prompts them to hunt down baboons. Some respondents mentioned that monkeys hide or run away from men, but do not respect women. Women will therefore not guard the farms from invading monkeys. One
particular farmer claimed that “baboons chase pregnant women and also beat-up children guarding after the crops”.

Some beliefs are however good for conservation. In the current survey, monkeys were reported as highly intelligent and even uneasy to hunt or trap. A respondent observed that they learnt about some medicinal plants from observing what monkeys fed on. Two respondents felt that monkeys are close to humans and hence shouldn’t be eaten. Another claimed that a neighbor had died of eating monkey meat. He alleged that the death had occurred after the neighbor ate raw monkey meat and liver. One other respondent also asserted that monkey meat when eaten would cause vomiting and diarrhea. Due to stigma associated with monkey meat, people who ate them were said to do it in hiding. They even shied off from seeking medication if they fell sick upon eating monkey meat. Others would feign causes of their illness and report anything other than monkey meat.

4.5.4.8 Other challenges in the study sites

Monkeys were reported to steal food from the granaries and even kitchens. Baboons were particularly said to be notorious since they are strong and break into such structures. There were claims that baboons chased small children when left alone and they also did not fear women. One respondent reported of an incident where a woman was killed and eaten by baboons. In addition to monkeys, porcupines, elephants,
leopards and hyenas were reported to be a great nuisance. Snakes were also said to roam in the area hence posing danger of snake bites to residents of these areas.
CHAPTER FIVE: DISCUSSION

5.1 Helminth prevalence

Mutara Ranch baboons in Laikipia country had the highest prevalence of helminth infections among the study sites probably because of high interaction of the baboons with people. Laikipia site being a ranch, the baboons in most cases depend on farms for food. In this locality, there are also tourist hotels which serve as an attraction to the baboons since they feed on left overs and some are fed by tourists. Feeding on left overs may expose humans to baboon parasites and in the process they also disseminate the parasites through feaces or other body discharges as previously described by Mafuyai et al. (2013). Moderate helminth infection of baboons in Tana River could be occasioned by the fact that being a reserve, the site is not fenced off and the baboons shared the same forest with humans. Humans cultivated in the forest which is on the banks of River Tana and they used the water for irrigation. This therefore necessitated the interaction with humans.

The low helminth prevalence in Tsavo baboons could be because it is a park and there is separation of human habitation with wildlife. There is an electric fence which kept off humans from entering the park and was expected to keep off wildlife from crossing to human habitations. However, baboons and other non-humans primates being very clever could find routes which were safe for crossing over to farms. These included jumping over from tall trees and river beds and furrows which allowed them to pass under the fence.
*Oesophagostomum* and *Strongyloides* had high prevalences in all sites among all parasites. It is however reported that *O. bifucum* strain which infects human is different from the one that infects NHPs (De Gruijter *et al.*, 2005; van Leishout *et al.*, 2005). These two parasites are still neglected and are not among the current list of Neglected Tropical Diseases (NTDs) listed by World Health Organization (WHO) yet they appear common and not given necessary attention. *Strongyloides* is termed as the most neglected among the neglected diseases yet it’s currently believed to infect an estimated 30—100 million people worldwide (Olsen *et al.*, 2009). The parasites’ high prevalence in the current study sites in baboons could be due to the high interactions between humans and the animals. If there are no interventions in humans then the parasites will cost the world more in future in control unlike if this was done now as the other helminthes are controlled. Currently, there is a campaign of Mass Drug Administration (MDA) for deworming by WHO with the aim to eliminate soil transmitted helminths that can be controlled through chemotherapy (WHO, 2006). Hopefully, as the other helminthes especially soil transmitted helminthes (STHs) which include *Trichuris*, Ascariasis and hookworms are controlled through Preventive Chemotherapy (PCT), these helminthes may also be cleared in human population.

The low prevalences of *Trichuris* and *S. mansoni* could be because they have been given attention in humans due to the fact that they are included in the WHO list of neglected diseases baboons (Mwinzi *et al.*, 2012). This therefore indicates that control of parasites in the human population is likely to reduce the disease prevalence in the
baboon population. The low prevalence of *Schistosoma mansoni* for instance which was reported in Tsavo baboons would be attributed to control measures in the human population which have also included vector control. Other measures such as preventive chemotherapy, an activity that is on course in school going children would equally be important since there would be no more source of infection from humans. Baboons have long been known to self-medicate against parasitic infections by use of herbs (Lozano, 1998). This implies that even if the animals cannot clear the parasites, they can maintain them to manageable levels. This is also evident in the intensity of infections which were mostly low in baboons from all the study sites. However, there will still be challenges if the baboons will continue harboring the parasites even in low intensities as they can still be a source of infection to humans especially in localities where the animals commonly interact with humans.

The effective response to treatment in both *Strongyloides* and *Oesophagostomum* in baboons indicated that their control in humans is achievable. Albendazole is still the drug regularly used for mass chemotherapy control as well as for individual treatment of most nematodes. This means with mass deworming against these parasites could also lead to their elimination from human populations. The main challenge remains in the wildlife and especially non-human primates and more so the baboon being sources of new infections due to their interaction with humans (Mafuyai *et al.*, 2013).
5.2 Hemoparasites

*Hepatocystis kochi*’s high prevalence in the current study could be a pointer to the endemicity of the parasite in some Kenya regions like the coast and Eastern region though it is not zoonotic. *Babesia microti* and *Entopolypoides macaci* are both zoonotic but lucky enough they were reported with low prevalences in the current study which could have indicated that the baboons too are able to fight off the infections. These animals may have acquired immunity due to the low dose infections as is seen in malaria infections which confer immunity after repeated infections (Gupta *et al*., 1999). This is probably why the infection intensities were very low in baboons in the present study hence the unlikelihood of developing clinical disease. These animals would therefore act as carriers of the zoonotic infections.

Polymerase chain reaction (PCR) is generally a better method of confirming positive cases especially with low intensity infection on blood smear. The disadvantage with the test is that it may also test positive among the already cured individuals. It is therefore advisable that PCR test be used alongside other tests like microscopy. *Hepatocystis kochi* has been reported to be endemic in baboons (*Papio anubis* and *Papio cynocephalus*) and African green monkeys (*Cercopithecus aethiops*) in East Africa (Leathers, 1978) yet it rarely causes clinical disease. There have been very few studies done on the parasite probably because it is not zoonotic.

Presence of the hemoparasites also indicated the presence of the vectors in the vicinity.
Since the *E. macaci* and *B. microti* are transmitted by ticks (Gleason and Wolf, 1974; Seethamchai *et al.*, 2008) control of ticks would be important in affected areas to cut the cycle of transmission. Proper diagnosis in humans is also important to differentiate between these infections (*B. microti* and *E. macaci*) and malaria which clinically present most similar signs. In the localities where the study was carried out, poverty is reported to be high which could contribute to lowered immunity of the individuals due to malnutrition hence increased chances of infection with these hemoparasites. In Tsavo, the vegetation was mainly made of shrubs while in Tana, the vegetation was of forest type along the river with shrubs off the banks both of which favor tick habitation (KWS, 2013).

The high prevalence of *H. kochi* also suggested the presence of *Culicoides* flies which are their vectors. These being biting flies, are blood suckers of humans and domestic animals even if they do not transmit any parasite. They could therefore interfere with normal feeding of livestock due to the irritation they cause hence malnutrition. Ixodidae ticks were collected from the baboons from Tana River and Tsavo. Humans were reported to go to the bushes and even into the parks for various reasons which also increase the risks of contact with these vectors and others that transmit zoonotic infections. In Tana River, farming was done on the Tana River banks, which was the same habitat for the baboons. This increased risk of coming in contact with vectors and environment contaminated with animal discharges such as stool, urine and saliva containing pathogens.
The baboons had erythematous lesions on the skin sites which could have been caused by ticks since the location of the lesions were mainly in body parts that are preferred by ticks. This would therefore be lesion left behind by ticks when they fell on the ground. This implied that even if animals did not have ticks, there are chances that the ticks were in the environment. These ticks were likely to be infective hence they could transmit the hemoparasites to humans. Fever was also evident in these baboons and the most likely cause of these would be the hemoparasites (Demessie and Derso, 2015). Lymph node enlargement was also associated to hemoparasites infection probably because of being systemic. The major parasites that would be implicated to these signs would have been H. kochi which had a high prevalence. These hemoparasites clinically present as malaria and they also have intraerythrocytic stages hence chances of misdiagnosis when blood smears are used. Since Tana River County is located in the coastal part of the country, it is endemic for malaria (Polley et al., 2006). There is need to relook at the diagnostic methods that are capable of identifying cases of zoonotic infections such as Babesia and Entopolypoides in health facilities. This is because with poor diagnosis, there are chances of abuse of malarial drugs in treating these infections which accelerates development of drug resistance. In addition, with the misdiagnosis, the pathogens would not be cleared in the population and transmission would continue.

5.3 Pathology and hematological changes associated with helminth infections

The low infection intensities concurred with the hematological picture witnessed in these animals since most of the parameters were within normal ranges. There were
cases of neutrophilia and lymphocytosis and no eosinophilia as is expected with parasitic infections. This could have implied that the low intensity infections were not strong enough to elicit an immune response and the parasites might have caused premunition to the baboons. These could also have been caused by other concurrent infections such as bacterial and viral infections which are also thought to immunomodulate helminth infections (Fox et al., 2000). Helminth infections also reduce immune responses to viral infections as seen in HIV infection in humans (Actor et al., 1993) hence even if they rarely cause death, they should be treated.

Treatment did not appear to cause much change in leukocyte differentials counts, but generally caused an increase in erythrocyte parameters which indicated a relief from some level of in apparent anaemia. It is known that a number of the parasites such as helminths and hemoparasites lead to depletion of nutrients and some are associated with anemia (Ezeamama et al., 2005). This could have been the case in the study where the treated baboons had elevation of erythrocyte parameters. It has also been reported that eosinophilia is not automatic with these infections (Ledesma-Soto et al., 2014) especially so with the low levels of infection as seen in the current study.

Helminth pathology varies depending on the life cycle. Not much had been done in baboons’ helminth pathology despite being very close physiologically to humans. It is also widely reported that most of the human helminth infections are zoonotic and infect baboons too. In the present study, there were various pathologies documented. The
baboons with high prevalence of *Oesophagostomum* had nodular lesions in large intestines which were caused by larval stage of the parasite inhabiting the sub mucosa. The helminth is known to cause clinical *Oesophagostomiasis* worldwide in domestic animals and focally and sporadically in humans (Makouloutou *et al*., 2014). The species implicated in both humans and non-human primates are *O. bufurcum*, *O. stephanostomum* and *O. oculeatum* (Makouloutou *et al*., 2014). The eggs once passed to the ground through feaces molt into rhabditiform L₁ in the environment. With good environmental conditions L₁ will molt into L₂ then to a filariform L₃. This is the infective stage which once ingested by the definitive host barrows into the sub mucosa of either small or large intestines where it is encysted. The L₃ then further molts into L₄ which then emerge into the lumen to molt into the adult stage (CDC, 2013). With the entry and exit of the parasite, there is severe destruction of the intestinal mucosa.

With the mucosa destruction, the parasites are likely to compromise of animal’s nutrition by interfering with digestion and absorption. In studies by Babu *et al*. (2011), they reported that *O. columbianum* infection caused hard, raised, slightly yellowish to greenish colored nodules with catarrhal inflammation in addition to destruction and desquamation of epithelial cells which is also noted in the present study. Local tissue sensitivity develops in animals due to repeated exposure to these parasites and the subsequent entry of the larvae into the sub mucosae which provokes an intense tissue reaction. The parasite is reported to produce secretions (Cephalic and oesophageal)
which are responsible for the chronic inflammation (Smith et al., 1992; Lapage, 1962) resulting in proliferation of the fibrous tissues.

*Strongyloides* was reported to have caused perivascular fibrosis in the lungs which could have been occasioned by their migration through the lung tissue. Its reported that *S. stercoralis* infections range from asymptomatic light infections to chronic symptomatic strongyloidiasis. The parasite have been reported to cause hyperinfection (uncontrolled multiplication) and its larvae disseminate to all internal organs especially in immunocompromised individuals (Olsen et al., 2009). The *Strongyloides* life cycle is more complex than that of other nematodes with its alternation between free-living and parasitic cycles, and its potential for autoinfection and multiplication within the host. It has two life cycles namely the free-living (non-parasitic cycle) and the parasitic cycle. In the free living cycle, the rhabditiform larvae is passed in the stool and can either become infective filariform larvae (direct development) or free living adult males and females that mate and produce eggs from which rhabditiform larvae hatch and eventually become infective filariform larvae.

The filariform larvae penetrate the human host skin to initiate the parasitic cycle. In the parasitic cycle, the filariform larvae in contaminated soil penetrate the human skin, and by various, often random routes, migrate into the small intestine. Historically, it was
believed that the \( L_3 \) larvae migrate via the bloodstream to the lungs, where they are eventually coughed up and swallowed. It is probably during this migration that it causes pathology as seen in the present study. There are also reports that \( L_3 \) larvae can migrate directly to the intestine via connective tissues (CDC, 2015). In the small intestine, they molt twice and become adult female worms. The females live threaded in the epithelium of the small intestine and produce eggs, which yield rhabditiform larvae. The rhabditiform larvae can either be passed in the stool, or can cause autoinfection (CDC, 2015).

In autoinfection, the rhabditiform larvae become infective filariform larvae, which can penetrate either the intestinal mucosa (internal autoinfection) or the skin of the perianal area (external autoinfection); in either case, the filariform larvae may disseminate throughout the body. To date, occurrence of autoinfection in humans with helminth infections is recognized only in *Strongyloides stercoralis* and *Capillaries philippinensis* infections. In the case of *Strongyloides*, autoinfection may explain the possibility of persistent infections for many years in persons who have not been in an endemic area and of hyper infections in immunosuppressed individuals (CDC, 2015). This may be an explanation to the high level of prevalence in baboons in the current study. This is therefore, a very important parasite to control and eliminate from human population. With the serious pathologies, the parasite if not controlled may be hard to eliminate from the human population.
*Trichuris* was implicated in intussusception of small intestines in the current study and this would be attributed to irritation of the intestinal mucosa. This is one of the parasites targeted for elimination by WHO being a preventive chemotherapy neglected tropical disease (PCT-NTD) (Brooker *et al.*, 2009). It has a direct life cycle. The unembroyonated egg is passed in the feaces into the soil where they develop into second stage cleavage and then embryonate to become infective. Following ingestion, the eggs hatch in the small intestines to release larva that mature and establish in the colon. The adult worm lives in the caecum and ascending colon where they are fixed in the wall with the anterior portion threaded into the mucosa (CDC, 2013). This parasite is implicated in intussusception in *Papio hamadryas*, another species of baboons (Hannesy *et al.*, 1994) as well as in humans. With this kind of pathology, there is usually strangulation of blood vessels in the affected area which causes tissue degeneration and necrosis in the parts supplied by these vessels.

In the present study, the necrosis seen in *Trichuris* infection could have been caused by strangulation of blood vessels following intussusception. Macrophages predominance would mainly mean that they were engulphing the dead cells which had suffered from hypoxia and lack of nutrients due to lack of blood supply (Shimizu *et al.*, 1996). Intussusception leads to blockage of the lumen hence no movement of food down the GIT. This can also lead to autointoxication since the feacal waste products are not removed from the body and soluble waste would leak into the body through the mucosa.
In addition, there is also distention of the abdomen due to accumulation of the ingesta in the GIT. Unless the intussusception is corrected naturally, then such animals are likely to die. Such animals being weak from ill health are likely to be an easy target for predators but in the absence of the predators then they will die on their own.

*Schistosoma mansoni* infection presented with parenchymal and periportal fibrosis in the liver as well as granuloma formation which could have been caused by migration of the parasite through the organ. Previous experimental studies on *S. mansoni* in the baboon reported varied pathologies including hepatic granuloma formation, periportal fibrosis, bile duct hyperplasia and angiogenesis in baboons (Farah *et al.*, 2000) some of which concur with most results in the current study. With these pathologies mirroring the picture seen in humans, baboons could be good models for study of these human infections. This is due to the fact that their physiology is closer to humans compared to other conventional disease models such as mice (Farah *et al.*, 2005). Since the disease is easily treated with Praziquantel and controlled by mass drug administration as well as hygiene practices, public education need to be scaled up to bring down its transmission.

### 5.4 Risk factors associated with zoonoses transmission

Tsavo was characterized by agro-pastoralists involving mainly maize, beans, cowpeas and sorghum cultivation, and livestock keeping which indicated diversification of crops cultivated. This would have been brought about by the crop raids especially on maize which is also the main crop in the country. Their proximity to Tsavo west and Chyulu
National parks often breeds inadvertent interactions with wildlife hence conflict. Tana River inhabitants live in clusters due to the insecurity but farm in the river banks where they would migrate to when the crops were at the stage prone to destruction by wildlife. The main source of water for Tana River residents was the river.

Low level of education was a contributor to other risks of zoonoses transmission as seen in Tsavo and Tana River. Tsavo residents practiced livestock farming and especially chicken farming, animals which are easily attacked by baboons. Tana residents mainly practiced maize farming, a crop that is also liked by baboons hence increasing chances of attacks. There was an association between illiteracy and consumption of livestock that died from unknown causes in Tsavo. Illiteracy in Tana was associated with lack of knowledge on zoonotic diseases which would perpetuate behaviors such as eating uninspected meat from wildlife including monkeys. This could in turn increase the risk of zoonoses transmission.

Land acquisition as a demographic feature indicated that people who inherited land had low earnings with most of them practicing subsistence farming hence reporting food insufficiency. These people also had low education levels mostly upto primary school level in Tsavo. This group of people is likely to suffer zoonotic infections as they are the most likely group with ignorance on zoonotic infections.
The main occupation in both Tana River and Tsavo was farming and this was associated with baboon crop raids and predation on livestock. The residents reported farm food insufficiency due to crop raids and this was attributed to certain types of farming practices. These included maize cultivation, goat and chicken rearing. With the food insufficiency, the inhabitants also feed on left overs from the crop raid and predation which the baboons had left behind. There was ignorance on zoonoses which could have been a contributor to the practice of left over consumption. There were also reports of inhabitants consuming monkey meat which could have been necessitated by the food insufficiency due to the destruction by baboons. People with single sources of livelihood which in the current study was farming would take all possible actions against the wildlife responsible for destroying their food source (Dickman, 2010).

Sharing water source is a major concern in zoonoses transmission. The current study found out that inhabitants shared water with baboons especially in Tana River where they used the river for irrigation as well as domestic use. Water is a major reservoir for pathogens especially those transmitted through the oral-feecal route. If crops are farmed near habitats of wildlife as is the case in Tana River where they farm in the river banks, this attracts crops raids hence a source of human-baboon interaction (Sillero-Zubiri and Switzer, 2001, Linkie et al., 2007). Most people are usually ignorant of risks associated with human wildlife interaction (Dickman, 2010).
Crop raid is a main a factor for zoonoses transmission as animals come in close proximity to humans. Crop raids by wildlife have been reported by Pimentel et al. (2005) and Perez and Pacheco (2006) with the animals being implicated in raiding stored food too. The crop raid was mainly necessitated by the people farming near the parks as was the case in Tsavo and in the Reserve as was the case with Tana. In the current study, the baboon was reported to be the most notorious in the act probably due to the strength of the animal compared to the smaller non-human primates in addition to its cleverness. The animal was also involved in predation of small livestock such as kids, lambs and chicken. The baboon has been reported as the major culprit in crop raids and livestock predation (Sillero-Zubiri and Switzer, 2001; Thirgood, et al., 2005). This would be a means of zoonoses transmission mainly when people feed on left overs. Body secretions such as saliva, nasal discharges, feacal and urine contamination left on these foods could contain zoonotic pathogens which eventually end up in humans.

Non-human primate consumption which was reported in low frequencies in the current study could be occasioned by poverty caused by the animals destroying crops and predating on livestock. The low frequency reported in the current study may not be the true picture since people might have feared the consequences from the government as wildlife hunting is illegal in Kenya. In the current study, the main culprit was the baboon which was reported as the most destructive. Hence the inhabitants could hunt them for revenge as reported in other studies that bush meat consumption is on the rise due to the increased human-wildlife conflict (Wang and Crameri, 2014). The residents
would also have committed these acts knowing that they could not be compensated by
the government, an act also seen in Tanzania (Sillero-Zubiri and Switzer, 2001). The
high ignorance on zoonotic diseases from NHPs was also a contributor to this activity as
most respondents were not aware of zoonotic infections from these animals.

Past similar studies by Siex et al. (1999) on human-wildlife conflict in Africa, similarly
pointed out crop raids and livestock predation as sources of the conflict. Due to
irregular patterns of weather often characterized by prolonged droughts, animals end up
straying from their habitats and seeking pasture, food and water in human settlements,
thereby exacerbating the conflict. Animals reported to perfect crop raids in the current
study included elephants, gazelles and baboons while livestock predation was blamed
heavily on baboons and leopards. Non-human primates are the commonest globally
(Sillero-Zubiri and Switzer, 2001), a case also seen in the current study.

In Laikipia, there were tourist hotels and the animals frequently fed on hotel left overs
and this was likely to be a source of their interaction with humans. As they feed on the
left overs, they defecate in these sites and their waste which contains helminth eggs is
carried away into surface water which would end up in water supplies to humans. In the
other study sites due to the proximity with humans, they can easily transmit and also
pick helminth oocysts. In Tana River for instance, inhabitants mainly use river water.
Activities such as washing, drawing water for domestic use, bathing, swimming
enhances the transmission. There are boreholes but people prefer river water which is taken without purification, the same water used by the NHPs.

Traditionally, communities adjacent to wildlife areas have occasionally derived their subsistence foods from wildlife through wildlife hunting for bush meat (Wolfe et al., 2000; Wolfe et al; 2004) which was also reported in the current study. Care for the wild Kenya, (2015) and Davis (2002) observed that hunting for bush meat has in the recent years evolved from subsistence to commercial. Worse still, efficient modern technologies of hunting continue to phase out traditional traps leading to faster decline in wildlife numbers (Hemson, 2004). Tourism is the back bone of Kenya’s economy and such declines impacts negatively on the country. It is also uncommon to find wildlife open markets as is seen in other African Nations, a fact that has helped save the wildlife population.

Besides issues of sustainability, significant health implications have been associated with hunting and slaughtering wildlife for meat. The 2002 Ebola outbreak in Gabon and the Republic of Congo was attributed to game meat consumption (Hewlett and Hewlett, 2008). Majority of the animals hunted in the current study belonged to the antelope family. It has been observed that people are shifting from hunting the more abundant animals like dik-diks and duikers due to their declining numbers to monkeys and other endangered species (Bush meat Crisis Task Force, 2003). The main reasons for hunting monkeys in the Tsavo and Tana River included hunger, revenge on destruction of crops
and livestock and curiosity for taste of monkey meat. Nevertheless, monkeys are in particular, reservoirs for zoonotic diseases due to their genetic closeness to humans (Bush meat Crisis Task Force, 2003). HIV/AIDS may have resulted from the transmission of chimpanzee-borne SIV (simian immunodeficiency virus) to humans, possibly from blood contact while killing and slaughtering bush meat (Gao et al., 1999).

Another source of interaction between humans and baboons was visitation of bushes for firewood and charcoal burning for their cooking and lighting which has been reported in other communities (Anderson, 2008). This was probably occasioned by poverty; hence the inhabitants could not afford alternative sources of energy such as gas. Charcoal burning was particularly on the increase especially in Tsavo. Farmers in the current study claimed that charcoal burning was the only way left for them to earn a living after agricultural activities are hampered by crop raiding and livestock predation. With such activities in the forest, human wildlife conflict is inevitable (Ladan, 2014). With increased human population, there is construction of roads and continued opening-up of areas that were once isolated. The trend is likely to worsen, accelerating human wildlife conflict.

Indigenous communities were characterised by high rates of poverty and malnutrition in comparison to other members of the society. Such are situations where neglected tropical diseases flourish most. This is because malnutrition means weaken immune system which is prone to infections. The inhabitants also had low literacy levels and
limited access to health services which also affects the control of the infections. Basic knowledge on control measures such as hand washing, use of portable water for drinking as well as avoiding infected water as is the case with schistosomiasis could lack in such communities. Most people are also likely to feed on leftover food after monkey raids and even consume monkey meat due to low literacy and also the high level of poverty.

Dry weather conditions also contributed to sharing of the few water points between monkeys and other wildlife, and in Tana, most of the inhabitants depended on river water which was also the sole water supply to wildlife. In both sites, tap water supply was very low which meant that they had to use other sources like wells and bore holes. Water points can be a crucial interface of zoonotic disease exchange (Thirgood et al., 2005). The current study established a high level of lack of awareness on zoonotic diseases transmitted from both bush meat in general and monkey meat consumption. This is in spite of information from the key informants alluding that Kenya Wildlife Service and community health workers (CHW) hold regular chiefs’ meetings commonly known as ‘barazas’ to educate the community on conservation matters and dangers of bush meat consumption.
CHAPTER SIX: CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

i. Prevalence of zoonotic helminths from the three study sites namely Tsavo, Tana River and Laikipia varied from 80.5% (*Oesophagostomum*) to 2.1% (*S. mansoni*) in Tsavo. Helminthes detected included *Oesophagostomum* which had highest prevalence in Laikipia baboons (80.5%) followed by *Strongyloides* (77.8%), *Trichuris* (27.5%) and *Enterobius* (14.8%) all in Tana River baboons. *S. mansoni* was only detected in Tsavo baboons (2.1%).

ii. The prevalence of hemoparasites detected varied in Tsavo and Tana River baboons and they included *Hepatocystis kochi* (90%, 87%) which is not infections to humans whereas, *Babesia* (10.8%, 16.7 %,) and *Entopolypoides* (8.7%, 5%) respectively. Laikipia baboons were all negative for hemoparasites.

iii. Hematology changes post treatment against helminthes indicated no significant changes in leukocyte differentials but there was a significant increase of all erythrocyte parameters post treatment except MCV though the parameters were all initially within the normal ranges.

iv. Tissue pathology due to helminth revealed nodular lesions with epithelial necrosis due to *Oesophagostomum* in large intestines. There was lung fibrosis due to *Strongyloides* infection and intussusception with epithelial necrosis in *Trichuris* infection. *S mansoni* infection revealed granuloma formation, parenchymal and periportal fibrosis in liver.
v. Some of the risk factors of zoonoses included sharing of river water, crop raids, livestock predation, consumption of left overs from crop raids predation, lack of knowledge of zoonoses and monkey meat consumption

6.2 Recommendations

i. Owing to the high prevalence *Oesophagostomum* and *Strongyloides* which are zoonotic, their control should be reconsidered. There is need to incorporate the two parasites in the WHO list of neglected tropical diseases so that they can be controlled like the other helminths currently in the list.

ii. Detection of *Babesia* and *Entopolypoides* in Tan River and Tsavo though at low prevalences indicates potential of humans contracting the infections since they are zoonotic. Integration of zoonoses training for hospital laboratory technicians is important for proper diagnosis especially for these hemoparasites which appear like *Plasmodium* in blood smears.

iii. Public education on regular deworming is important to reduce cases of anemia, and other pathologies due to helminths which may lead to poor performance of individuals. Since there is ongoing mass drug administration, parents should be sensitized to allow their children to take the drugs.

iv. Public health education on zoonoses and hygiene is also important in reducing the risk of zoonoses transmission from baboons at the human wildlife interface. Improving compensation due to losses caused by baboons would be vital in
reducing the consumption of left overs from crop raids hence reduce zoonoses transmission. The government can also consider relocating inhabitants from park borders to reduce the interaction between the baboons and humans.

v. Future studies can focus on the herbs used by baboons in treatment of infections in the wild. Studies can also focus on premedication which appears to favour baboons since they have low doses of the parasites and they are able to stay healthy. This appears like a kind of stimulation of the immune system, which can be studied in humans especially for hemoparasites like malaria that do not yet have a vaccine.
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APPENDICES

APPENDIX I: Questionnaires

RESPONDENTS QUESTIONNAIRE

Questionnaire No______________________________

SECTION 1: Household Data

Please tick (√) appropriately

To determine the risk factors associated with transmission of the zoonoses at the NHP-human interface

Study site ___________________________Household number __________________________

Respondent Name__________________________

1) Respondent’s gender: □ Male □ Female

2) Age (years): ________________________

3) Marital status: □ Single □ Married □ Divorced/separated □ Widowed

4) Gender of household head: □ Male □ Female

5) Number of children in the household (HH): ____________________________

6) Education level of HH: □ None □ Primary □ Secondary □ Tertiary

7) Occupation of HH: □ Farmer □ Formal employment □ Other (specify) _______

8) Residence: Division________________________ Location________________________

9) Length of stay in current residence: Years__________ Months_____________________

SECTION 2: Socio-economic Data

Please tick (√) appropriately

10) Describe your land ownership?

□ Leased □ Bought □ Inherited □ Other (specify) _______

11) What is the average size of your plot/land?
Less than 1 acre      1-5 acres      More than 5 acres

12) Describe your house ownership?
     Own      Rented      Other (specify) ___________

13) If rented, how much rent do you pay (Kenya shillings)?

14) What are the walls made of (What about roof)?
     Timber      Mud      Iron sheets      Stone      Other (specify) (specify)

15) What is the size of the house?
     1 Room      2 Room      1 Bedroom      2 Bedrooms      3+ Bedrooms

16) What is the average household income (Kenya shillings/month)?
     Less than 3,000      3,001-6,000      6,001-10,000      More than 10,000

17) What type of agricultural activity do you practice?
     Crop cultivation      Livestock keeping      Mixed crop-livestock enterprise

18) Who is mostly responsible for agricultural activities in the farm?
     Man      Woman      Both      Other (specify)

19) List the type of crops you cultivate?

20) For what purpose do you practice crop cultivation?
     For subsistence use      Commercial undertaking      Both

21) Does crop cultivation contribute enough food for the household? Yes No

22) If no, where do you get more food from?
     Purchase (market/grocery)      Relatives/friends      Welfare/NGO support
     Other (specify)

23) What mode of cultivation do you employ on your farm?
     Machine use      Manual

24) Which type of livestock do you keep?

Please tick (√) appropriately

<table>
<thead>
<tr>
<th>Large livestock</th>
<th>Cattle</th>
<th>Sheep</th>
<th>Goats</th>
<th>Pigs</th>
<th>Other (specify)</th>
</tr>
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<tbody>
<tr>
<td>Number</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rearing system</td>
<td></td>
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</tr>
</tbody>
</table>
N.B Rearing system fill: 1 = within own compound, 2 = Free range, 3 = Both within own compound and free range,

25) Is there a recent history of disease outbreak that affected your livestock?
☐ Yes ☐ No

26) If yes, when did the outbreak occur?
☐ Less than 1 year ago ☐ 2-3 years ago ☐ More than 3 years ago

SECTION 3: Risk Factors

27) Where do you get water for the household and animals from?
☐ Tap water ☐ Borehole ☐ Well ☐ River/stream

28) Do you wash fruits/vegetables before cooking? ☐ Yes ☐ No

29) Have you ever seen a monkey? ☐ Yes ☐

30) If monkeys come into your farm to crop raid? ☐ Yes ☐ No

31) If yes which species is notorious for this?..........

32) Do monkeys predate on your livestock? ☐ Yes ☐ No

33) If yes which species is notorious in this?..........

34) Do people eat the crop or livestock remains of after monkeys attacks or raids? ☐ Yes ☐ No

35) Do people in your area hunt wild animals for bush meat? ☐ Yes ☐

36) If yes which species are commonly hunted?..................

37) What is the main reason for bush meat hunting, subsistence, commercial, revenge pleasure other

38) Monkey meat is currently becoming popular bush meat in Kenya, do people here consume monkey meat? ☐ Yes ☐ No

39) Do you know of people who eat monkey meat? ☐ Yes ☐ No

40. What reasons do they provide for eating monkey meat? ☐ Hunger ☐ Taste curiosity ☐ revenge for crop destruction ☐ Medicinal ☐ Cultural ☐ Other reasons

41) Which species of monkeys do people here prefer for bush meat?..........

42) How do you prefer to consume your meat? ☐ Thoroughly cooked ☐ Partially cooked ☐ Raw ☐ Roasting

43) Do those people who hunt monkeys for meat in the area share or sell it to other people? ☐ Yes ☐ No

44) Have you seen monkey meat sold? ☐ Yes ☐ No

45) If yes where? ☐ Market ☐ Bush ☐ hawked in homesteads

46) Do you employ any preservation methods for your meat? ☐ Yes ☐
47) If yes, how do you preserve meat? □ Freeze □ Smoke □ Other (specify) □

48) Have you come across someone who has become ill after consumption of monkey meat? □ Yes □ No

49) Do you know of people who have consumed meat from livestock which die of known or unknown diseases? □ Yes □ No

50) Are you aware of any diseases caused by bush meat consumption? □ Yes □ No
   i) If yes which ones ....................................................

51) Have you ever seen a sick monkey? □ Yes □ No
   i) If yes, how did you tell it is sick? ........................................

52) Have you a monkey which died on its own? □ Yes □ No

53) How did you handle the carcass ...........................................

54) Is there anything else you know about bush meat or monkeys that you would like to share?

Thank you for taking time to participate in this interview

KEY INFORMANT QUESTIONNAIRE

NAME: ________________________________

1. List the type of meat eaten by people in this area? ........................................

2. Where do they get the meat from? ..............................................................

3. What types of monkeys are found in this area? ...........................................

4. Where do these monkeys come from? .......................................................

5. Do the monkeys raid the crops? Yes □ No □

6. How do people control these raids? .........................................................

7. What are your opinions on the monkeys invading people farms? ..............

8. Have you seen people here eat monkey meat? Yes □ No □

9. If yes, how often do they consume the meat? Daily □ Weekly □
10. What reasons can you provide for people eating monkey meat? …………………

11. How do they prefer to consume the meat? …………………………………………

12. Have you seen monkey meat sold? Yes □ No □

13. If yes where? Market □ Bush □ hawked in homesteads □

14. Do people employ any preservation methods for the meat? Yes □ No □

15. If yes, how do you they preserve meat? Freeze □ Smoke □ Other (specify)____________________________________________

16. Have you seen people experience any problem from consuming monkey meat
   Yes □ No □
   i) If yes please specify what problem____________________________________________

17. Do monkeys predate on you livestock? Yes □ No □

18. If yes which species is notorious for this?_____________________________________

19. What are your opinions on bush meat consumption/hunting?

20. Are there any activities to prevent bush meat hunting

21. When monkeys are killed is it reported

22. Is there any surveillance on animal security?

23. Is there any form of sensitization on monkey conservation?

24. Is there any activity to enhance human - wildlife coexistence e.g. community conservation?

25. Are there any compensation mechanisms for destruction caused by monkeys?

26. Are community members punished for hunting/killing monkeys?

27. Are you aware of any diseases caused by consumption of monkey meat?

28. What are your recommendation regarding bush meat trade?
APPENDIX II: Baboon fecal egg count protocol

Fecal Sedimentation Protocol

Note: Until step 10, this protocol is very similar to the flotation protocol above.

i. Homogenize the fecal sample thoroughly with a stirring stick. This step is important to make sure that the parasite eggs are uniformly distributed in the sample.

ii. Place a plastic fecal sample jar on the scale and tare the jar.

iii. Weigh out 2 grams of fecal sample into the plastic fecal sample jar and remove from the scale.

iv. Add 12 ml of water to the sample and mix with a stirring stick until the mixture becomes a thin brown slurry. Mix the sample again thoroughly with the stirring stick.

v. To remove large debris from the sample, swirl the sample to suspend the sediment and pour the slurry through a tea strainer into another fecal sample jar.

vi. Swirl the filtrate to suspend the sample and pour the filtrate into a 15 ml conical tube in a test tube stand. If the sample volume is less than 14ml, fill the tube to the 14 ml mark with water (with a pipette).

vii. If you are running multiple samples, add water to the tubes so that they all have the same volume of liquid (i.e., 14ml). If necessary, make a tube of water to balance the centrifuge.

viii. Cap the samples and centrifuge them at 1500 rpm for 10 minutes.
ix. Pour off the supernatant.

x. Add 750ul of clean tap water to the sample using a mechanical pipette.

xi. Add 500ul of concentrated sugar solution to help prevent the slides from drying out under the microscope.

xii. Mix the sediment, water and sugar solution thoroughly using a disposable plastic pipette.

xiii. Place two small drops of sediment on the slide, and scan under 10X power to confirm the presence or absence of nematode eggs. Start at one corner of the slide and move upwards in a line counting eggs. Note that unlike the float slide, the eggs will mostly be in the focus plane just above the slide surface (i.e., the eggs will sink to the bottom), so make sure you are focused at the correct level while you are counting. When you’ve finished one row, slide over one field of view and count the next row until you finish the slide. Count the number of each type of parasite eggs you see in the slide and record them on the sample sheet. For the first 10 of each parasite type, measure the length and width of the egg using the ocular micrometer. Do not measure eggs positioned at an angle so you can’t get an accurate measurement of length or width.

**Saturated sugar solution preparation**

To prepare this solution, weigh 454g of sugar and dissolve this in 355ml of hot water. Add sugar to hot water over a low heat and stir. Measure the specific gravity after the solution cools (SG ~ 1.27). To prevent mold growth, add 2ml of 37% formaldehyde
APPENDIX III: Ethical approval

INSTITUTIONAL REVIEW COMMITTEE (IRC)

FINAL PROPOSAL APPROVAL FORM

Our ref: IRC/15/11

Dear Dr Mercy Akinyi

It is my pleasure to inform you that your proposal entitled “Epidemiological Surveillance of Zoonotic Pathogens of Non-Human Primates in Tana River Primate Reserve and Tsavo National Park” has been reviewed by the Institutional Review Committee (IRC) at a meeting of 29th November 2011. The proposal was reviewed on the scientific merit and ethical considerations on the use of animals for research purposes. The committee is guided by the Institutional guidelines (e.g. S.O.Ps) as well as International regulations, including those of WHO, NIH, PVEN and Helsinki Convention on the humane treatment of animals for scientific purposes and GLP.

This proposal has been approved and you are bound by the IPR Intellectual Property Policy.

Signed [Signature]
Chairman IRC

Signed [Signature]
Secretary IRC

Date: 29th November 2011

[Stamp: INSTITUTE OF PRIMATE RESEARCH
INSTITUTIONAL REVIEW COMMITTEE
P. O. Box 24481-00502 KAREN
NAIROBI - KENYA
APPROVED 29/11/2011]
APPENDIX IV: Tissue processing by paraffin wax infiltration method

Dehydration
i. Cut tissues to be processed into small pieces (2mm) from each organ tissue submitted for
ii. Processing using a scalpel blade.
iii. Transfer the tissue into a tissue processing capsule (basket) together with its label.
iv. Put the tissue processing capsules into the different solvents using a pair of forceps serially as per the schedule given below.
   80% ethanol 1 hour
   95% ethanol 4 hours
   95% ethanol 2 hours
   100% ethanol 2 hours
   100% ethanol 1 hour
   100% ethanol 1 hour

Clearing
After the above steps, clear the tissues by passing them through three changes of Toluene (analytical research grade) at one (1) hour intervals.

Infiltration
i. Infiltrate the tissue by transferring them into molten wax bath which is then put in a vacuum oven at 58-60°C for one hour
ii. After one hour transfer the tissues into another wax bath for and treat as above.

Casting /blocking
i. Put a little molten paraffin wax into a pre-warmed mold.
ii. Place the tissue to be molded into this mold using a warm pair of forceps adjust it such that it is at the center of the mold.
iii. Carefully Put a labeled embedding ring on top and transfer the mold on a cold surface for
iv. wax block to solidify. Add more wax and allow the block to cool.
v. Remove the mold and store the blocks at 4°C as they await trimming and sectioning.

Note: if air bubbles are presenting a tissue block, the block can be melted and the tissue re-infiltrated in the vacuum oven for up to one hour and re-casted.

Trimming
i. Put all tissue blocks to be trimmed on ice in an ice container.
ii. Carefully fix the trimming knife onto the microtome and adjust it to cut 10μ sections.
iii. Fasten the tissue block firmly onto the microtome stage.
iv. Cut the blocks just to expose the tissue and keep the trimmed blocks at 4°C as they wait.
v. sectioning.

Sectioning
i. Fill water bath with water and add 40mg of gelatin (section adhesive) and set it at 43-46oC.
ii. Put all tissue blocks to be sectioned on ice in an ice container.
iii. Carefully fix the sectioning knife onto the microtome and adjust it to cut 6μ sections.
iv. Fasten the tissue block firmly onto the microtome stage (tissue block holder).
v. Cut the blocks steadily in order to get good ribbons. Once good ribbons have been made, transfer them to float on the warm water bath using dump carmel hair brushes.
vi. Carefully pick the floating sections onto a clean microscope slide.
vii. Wipe the underside of the slide and label the slide with animal number, name of the tissue etc. using a graphite pencil.
viii. Arrange the paraffin wax preparations (slides) in a vertical staining rack.
ix. Transfer the slide rack into a dust free oven preset at 60oC for 30-60 minutes or at 45-50oC overnight.
APPENDIX V: Haematoxylin and eosin staining for tissue sections

- Remove the tissues from the formalin (preservative)
- Deparaffinize tissues with Xylene 5 minutes

Hydrate the sections:
  i. 100% ethanol for 5 minutes
  ii. 95% ethanol for 5 minutes
  iii. 80% ethanol for 3 minutes
  iv. Wash with running tap Water 1 minutes
  v. Stain with Harris haematoxylin 10-15 minutes
  vi. Wash with running tap Water 1 minute
  vii. Decolourize with 0.5% acid alcohol 2 dips
  viii. Wash with running tap Water 1 minute
  ix. Blue in 2-3% lithium carbonate (ammonia water) 45 seconds
  x. Wash with running tap Water 1 minute
  xi. Counter stain with Eosin 5 minute

Dehydrate:
  i. 95% ethanol -14 dips
  ii. 95% ethanol -14 dips
  iii. 100% ethanol - 14 dips
  iv. 100% ethanol – 14 dips

Clear with: Xylene
Xylene -14 dips

Mount with DPX
APPENDIX VI: DNA extraction and polymerase chain reaction

DNA EXTRACTION PROTOCOL USING ZYMO –RESEARCH KIT

i. 40 µl of genomic lysis buffer was added to 100µl blood then mixed by vortexing for 5 seconds then left to stand for 10 minutes at room temperature

ii. The mixture was then transferred to a zymospin column in a collection tube and centrifuge at 1000g for 1 minute. The collection tube with the flow through was then discarded.

iii. The zymospin column was transferred into a new collection tube. To the column, 200 µl of prewash buffer was added and column centrifuged at 10,000g for 1 minute again

iv. The column was again transferred to a new collecting tube and 500 µl of the gDNA wash buffer added to the column. The column was then centrifuged again at 10,000g for 1 minute

v. The spin column was then transferred to a micro-centrifuge tube and 50 µl DNA elution buffer added. This was incubated for 4 minutes at room temperatures then centrifuged at 10,000g for 30 seconds to elute the DNA.

vi. The spin column was then discarded and the DNA in the micro-centrifuge tube stored at -20°C awaiting PCR.

VISIOLIZATION OF PCR PRODUCTS

i. 2% of the electrophoresis agar rose gel was prepared as follows by adding 2g of agar rose powder to 100ml TAE buffer.

ii. This was then heated for 1 minute and 45 seconds in a microwave to aid in dissolution

iii. 5ml of Ethidium bromide was then added to the hot solution and swilled to dissolve the ethidium

iv. The solution was then poured on the electrophoretic tank and allowed to cool

v. The wells were made using a comb
vi. Thermo scientific orange DNA loading dye (Inqaba – South Africa) was mixed with both the 100 base pair ladder and samples on parafilm paper at volumes of 5 µl of the sample and 5 µl of the dye.

vii. 100bp ladder was placed in the first well to give the parasite genome size and the samples placed on the remaining wells.

viii. Electrophoresis was run on the gel at 100 volts for 1 hour and 30 minutes then visualized under UV light then photographed.