MOLECULAR DETECTION OF ZOONOTIC HELMINTHS INFECTING NON-HUMAN PRIMATES IN KENYAN URBAN CENTRES

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DECLARATION AND RECOMMENDATION

Declaration

This research thesis is my original work and has not been submitted or presented for examination in any institution for award of any degree.

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Recommendation

This thesis has been submitted with our approval as supervisors for examination according to Egerton University regulations.

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To Lydiah G. Mwangi

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ABSTRACT

Natural infections with soil transmitted helminthic nematodes occur in non-human primates (NHPs) and have the potential to cross primate-species boundaries and cause diseases of significant public health concern. Consequently, the identification and molecular characterisation of worms infecting NHPs living in proximity to humans is key to surveillance and control of zoonotic nematodes originating from wildlife. Despite the presence of NHPs in most urban centres in Kenya, there is little information on genetic characterisation of circulating soil transmitted parasitic nematodes and the role of NHPs as réservoirs in urban settings. Therefore, a cross sectional survey was undertaken, and polymerase chain reaction coupled with high-resolution melting (PCR-HRM) analysis and microscopy applied for detection of parasitic nematodes infecting free-ranging common NHPs found within selected urban centres in Kenya namely Mombasa, Kisumu, Kakamega and Murang'a. Eighty-six faecal samples from *Chlorocebus aethiops* (African green monkey, AGM, n=41), *Papio anubis* (Olive baboon, n=30), *Cercopithecus mitis* (blue monkey, n=5) and *Cercopithecus ascanius* (red-tailed monkey, n=10) were collected by rectal swabbing. Combination of parasitological identification and PCR-HRM detection of helminthic nematode revealed a rich diversity of helminths including *Oesophagostomum stephanostomum* and *Oesophagostomum bifurcum*. PCR-HRM analysis of ITS-2 rDNA gene generated two distinct profiles corresponding to *O. stephanostomum* and *O. bifurcum*, and the results was further confirmed by sequencing. Two AGMs were infected with *O. stephanostomum* and a total of 11 NHPs were co-infected with *O. stephanostomum* and *O. bifurcum.* Phylogeny analysis of the parasites' sequences showed *O. stephanostomum* and *O. bifurcum* clustered with reference human isolates suggesting close genetic similarities. Overall, this study demonstrated the suitability of PCR-HRM as a non-subjective method for rapid detection and differentiation of nodular worms by generation of distinct melt curves for each worm species. To our knowledge, we report, for the first time, the co-infection of nodular worms in NHPs, which further informs on the epidemiology of these zoonotic nematodes in Kenya urban centres. Finally, results confirming that different species of NHPs harbour potentially zoonotic nematodes within urban environs will inform on broader helminth control strategies in endemic tropical countries like Kenya.

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LIST OF ABBREVIATIONS AND ACRONYMS

AGM	African green monkey
AIDs	Acquired immune deficiency syndrome
BLAST	Basic local alignment search tool
CWW	Children without worms
DALYs	Daily adjusted life years
DEC	Diethylcarbamazine
EIDs	Emerging infectious diseases
EPG	Egg per gram
FEC	Faecal egg count
GIT	Gastrointestinal tract
HAART	Highly active antiretroviral therapy
HAT	Human African trypanosomiasis
HIV	Human immunodeficiency virus
HRM	High resolution melting
HRV-IV	Hyper variable region IV
ID	Infectious diseases
IgE	Immunoglobulin E
IPR	Institute of primate research
ITS	Internal transcribed spacer region
IVM	Ivermectin
KWS	Kenya wildlife services
LF	Lymphatic filariasis
MDA	Mass drug administration
MERS	Middle east respiratory syndrome
MIF	Merthiolate iodine formaldehyde
NECT	Nifurtimox-eflrnithine combination treatment
NHPs	Non-human primates
NTDs	Neglected tropical diseases
PCT	Preventive chemotherapy
PCR	Polymerase chain reaction
PZQ	Praziquantel
Re-EIDs	Re-emerging infectious diseases
SARS	Severe acute respiratory syndrome

SIV	Simian immunodeficiency virus
SSA	Sub Saharan Africa
STHs	Soil transmitted helminth
TB	Tuberculosis
VL	Visceral leishmaniasis
WASH	Water sanitation and hygiene
WHO	World Health Organisation

CHAPTER ONE

INTRODUCTION

1.1. Background Information

Soil-transmitted helminth (STH) infections, also known as helminthiases, caused by parasitic intestinal worms, affect millions of poor and most deprived people, domestic animals and wildlife. They disproportionately affect the most impoverished population in the world, most of who inhabit the tropics. They are a part of neglected tropical diseases (NTDs), a category of infectious diseases (IDs) that occur in tropical and subtropical regions and in arctic regions, North America, and Europe (Hotez *et al*., 2008; Hotez, 2010). Globally, IDs resulting from viral, bacterial, parasitic, protozoan and helminths infections cause approximately 15 million deaths annually (Morens & Fauci, 2013).

Neglected tropical diseases (NTDs) include diseases that result in few deaths but impair health and productivity and are worsened by poverty. Examples include those caused by bacteria (e.g. Buruli ulcer and leprosy), viruses (dengue and chikungunya), protozoa (sleeping sickness and leishmaniasis) and helminths (STHs and cysticercosis) among others. They represent the most common illnesses of the world's poorest people (WHO, 2010). They are more than 17 in number and co-occur in 149 countries, inhabited by more than 4 billion people (Lustigman *et al*., 2012). They cause enormous health and economic burden, especially in Sub Saharan Africa (SSA). Owing to their huge health impact, there is a need for their improved control. NTDs control can be enhanced by improving our understanding of their transmission dynamics, including the role played by wildlife reservoir hosts including non-human primates (NHPs).

Wild free-ranging NHPs are potential hosts and/or sources of some important emerging/re-emerging zoonotic diseases in humans (Wolfe *et al*., 2007; Gillespie *et al*., 2008). The anthropoid primates (gorilla, and chimpanzees) and simian NHPs (monkeys) have similar physiology and genetic characteristics. Humans are thus vulnerable to infections by the same species of pathogens which have the potential to cross primate-species barriers (Wolfe *et al*., 2007). In addition, common habitats and the close evolutionary relationship between NHPs and humans pose a threat for cross-transmission of pathogens, including gastrointestinal (GIT) worms (Ghai *et al*., 2014). In fact, NHPs are infected and harbour a variety of intestinal parasites which include helminths. Helminthiases caused by nematodes (roundworms), trematodes (flukes) and cestodes (tapeworms) are diseases of public health concern in Kenya and Africa

at large. In Kenya, STH infection prevalence is highest at the coastal, central and western Kenya regions, with more than 10 million people being infected and approximately 16.6 million people being at risk (Mwandawiro *et al*., 2019).These parasites are NHPs-infective and despite the NHPs potential role as reservoirs, molecular studies on the epidemiology of zoonotic nematodes infecting Kenyan monkeys found within urban and peri-urban habitats are scarce.

Helminthiases caused by STHs include infestation by parasitic nematodes such as roundworm (*Ascaris lumbricoides*), whipworm (*Trichuris trichura)* and hookworms (*Necator americanus* and *Ancylostoma duodenale*). The STHs primarily infect individuals through water and/or food contaminated with eggs and larvae that are shed in faeces of an infected animal and/or human (Mohamed *et al*., 2016). Infections by helminths is a burden to the public health sector of tropical countries, affecting more than one billion people worldwide (Hotez *et al*., 2005; Bethony *et al*., 2006; Basuni *et al*., 2011; Jourdan *et al*., 2018). The infection is ubiquitous among NHPs as well as domesticated animals, and the worms can live in mammalian hosts for many years or decades, presenting a complex zoonotic disease transmission dynamic that complicates control and management.

Most NHPs are naturally infected by parasitic intestinal worms including *Strongyloides* sp., *Oesophagostomum* sp., *Trichuris* sp., *Streptopharagus* sp., *Enterobious* sp., *Bertiella* sp., and *Dicrocoelliidae* sp., (Gillespie, 2006; Thanchomnang *et al*., 2017). In Kenya, free-ranging baboons (*Papio cynocephalus* and *Papio anubis)* were found to harbour *Strongyloides* sp., *Physeloptera* sp., *Trichuris* sp., *Oesophagostomum* sp., and *Enterobious* sp., (Mbora & Mcpeek, 2009). These parasites are also human-infective, suggesting NHPs could play a role in the transmission dynamics of human worm infections. However, the presence of nodular worm in NHPs in Kenyan urban centres remains unknown, despite the potential role NHPs could play in human infections.

In Kenya, NHPs represent one of the most diverse taxonomic groups of wild mammals. Taxonomically, they fall under three families encompassing approximately 24 species (Groves, 2001; Grubb *et al*., 2003). The family *Cercopithecidae* is the largest with 16 species. African green monkeys (AGMs) and olive baboons, in this family, are the most widely distributed and commonest monkeys in Kenya. AGMs are terrestrial and commonly found in savannah, woodland, lakeshore, and coastal forests. Blue monkeys (Sykes) are arboreal and are widespread throughout forested regions in Kenya. Baboons are majorly terrestrial and adaptable opportunists that do well almost anywhere, including near human habitation. They are found in a wide

variety of habitats, from forest through grasslands to semi-arid areas (Eley, 1989). NHPs are ideal experimental models in biomedical research owing to their phylogenetic resemblance to humans (Zhang *et al.*, 2014). In Kenya, they are widely distributed and regarded as pests in most agricultural zones and villages and as wildlife in urban parks such as in Nairobi arboretum and Kisumu Impala Park.

Helminths parasite control involves various methods, including chemotherapy and improved sanitation. Anthelminthic drugs used include Praziquantel, Benzimidazole, and Ivermectin, and they greatly reduce the prevalence and intensity of infections (Onkanga *et al.*, 2016). Improved sanitation as a control strategy involves access to clean water, sanitation and hygiene (WASH) practices (Freeman *et al*., 2013), which disrupt infection (or re-infection) and transmission. The two control strategies, when applied, are effective. However, better sanitation provision, which is a responsibility of national governments, is a challenge in developing countries where the diseases are endemic. In addition, NHPs sharing habitat with human limit these control strategies by acting as reservoirs of these pathogens. Consequently, the benefits of current control strategies are jeopardised, a state that needs to be addressed.

In order to formulate effective control measures, accurate diagnosis and genetic characterization of nematodes is vital. Apart from conventional coproscopical methods for the identification of STHs, (Knoop *et al.*, 2014), the highly sensitive polymerase chain reaction (PCR) targeting the ribosomal internal transcribed spacer 2 (ITS2) gene of nematodes (Ghai *et al.*, 2014) has emerged as a superior technique. Additionally, real-time high-resolution melting (HRM) analysis, a probe-free post-PCR analysis, allows direct characterisation of PCR amplicons by measuring the fluorescence of intercalating dye in the process of separating the double-stranded DNA in a one-step closed-tube method (Reed *et al.*, 2007). PCR-HRM provides a useful tool for rapid identification and differentiation of species in the same genus with an added advantage of data storage and analysis capabilities *in silico*. This molecular technique has been effectively used to genotype gastrointestinal parasites without the use sequencing methods or electrophoresis (Rojas *et al*., 2017) making it a robust method for answering epidemiological questions that underpin pathogen surveillance and control programs (Villinger *et al.*, 2017).

Nematodes in the genus *Oesophagostomum* are considered endemic to west African countries. Its transmission potential between human and NHPs under natural conditions has been a source of considerable debate. In Ghana, the identification of genetic differences among *Oesophagostomum* nematodes infecting humans and population of baboons in the same region

suggested that the parasite is not commonly cross transmitted. However, a novel *Oesophagostomum* clade infecting human and five sympatric species of NHPs was recently described in Uganda. While great apes harbour *O. stephanostomum*, two cases have been reported in humans. This underscores the importance of local scale research for zoonosis risk and epidemiology of oesophagostomosis and the role of NHPs as potential reservoirs of the infection in Eastern Africa. This study aimed to determine distribution of zoonotic nematodes and species diversity of *Oesophagostomum* worm in NHPs found in selected urban centres in Kenya. As animals of diverse habitats, urban-restricted free-ranging NHPs pose a significant threat to emergence of zoonoses of public health importance (Akinyi *et al*., 2019) due to close and frequent interactions. Previous surveys on helminths have focused on NHPs within wildlife reserves and rural forest habitats (Ghai *et al*., 2014; Akinyi *et al.*, 2019; Obanda *et al*., 2019) leaving information gap on helminth zoonoses originating from free-ranging NHPs within urban and peri-urban centres. Insight of a study that addresses this will be important: firstly, informing on commonly occurring STHs in NHP and their role in maintaining transmission to humans; secondly, inform on appropriate application of the current control strategies especially for eradication and elimination target sets; and finally, formulation of new health policies for control of helminthiases.

Therefore, this study utilised parasitological and molecular techniques to detect the presence of STHs and characterise nodular worm in rectal swabbed faecal samples from *Chlorocebus aethiops* (African green monkey, AGM), *Papio anubis* (Olive baboon), *Cercopithecus mitis* (blue monkey) and *Cercopithecus ascanius* (red tailed monkey). Nodular worm infection is currently diagnosed by coproscopy which is unspecific and requires cultivation of eggs to L3 larvae via faecal cultures to differentiate them from hookworm egg. Animals were trapped in Kisumu, Mombasa, Murang'a and Kakamega, urban and peri-urban centres in Kenya. Phylogenetic analysis of the ITS2 rDNA regions of *Oesophagostomum* spp revealed that infections with *Oesophagostomum* species, considered endemic in West African countries (Togo and Ghana) also occur in Kenya, a first molecular observation in urban inhabiting NHPs. In addition, various zoonotic STHs infecting urban inhabiting NHPs were detected, suggesting potential risk for cross-transmission in human. These NHPs can thus act as reservoir and potential source of human helminth in densely populated urban centres allowing infections and/or re-infection. Therefore, implementation of effective control measures for STHs infections should factor the potential role of NHPs and other hosts as reservoirs of these zoonotic infections.

1.2. Statement of the Problem

Helminths are among the most common infectious agents of humans, NHPs, and livestock in developing countries. Their burden on the host is huge since they can last months, years and even decades in hosts, which complicates their control. In addition, the zoonotic nature of their transmission potentially limits the effectiveness of current control strategies adopted for human helminthiases. Data on zoonotic helminthic parasites of urban inhabiting NHPs are still limited, especially on common and widely distributed olive baboons, African green monkey and blue monkey, which are hosts to a range of gastrointestinal parasites. These animals share habitat with humans and may be involved in continued maintenance of zoonotic helminths in an urban ecosystem. However, there is little knowledge on distribution and species diversity of nodular worm in free-ranging NHPs in urban centres where ecosystems are shared between NHPs and humans. Such knowledge will be important in ensuring effective deployment of appropriate helminthiases control strategies.

1.3. Objectives

1.3.1. General Objective

To identify and characterise the zoonotic soil transmitted helminths in common free-ranging NHPs inhabiting selected urban and peri-urban centres in Kenya.

1.3.2. Specific Objectives

i. To identify and determine the distribution of soil transmitted helminths in free-ranging *Chlorocebus aethiops* (African green monkey, AGMs), *Papio anubis* (Olive baboon), *Cercopithecus mitis* (blue monkey) and *Cercopithecus Ascanius* (red tailed monkey) in Kisumu, Kakamega, Mombasa and Murang'a counties.
ii. To determine the species diversity of nodular worm in the free-ranging NHPs.
iii. To assess the effects of demographics covariates on the patterns of parasitism.

1.4. Null Hypotheses

i. There is no significance difference in distribution of zoonotic soil transmitted helminths in various free-ranging NHPs in Kenya.
ii. There are no diverse nodular worms' species in free-ranging NHPs.
iii. Demographic covariates do not affect the patterns of parasitism.

1.5. Justification

Free-ranging wild, *Chlorocebus aethiops* (African green monkey, AGMs), *Papio anubis* (Olive baboon), *Cercopithecus mitis* (blue monkey) and *Cercopithecus Ascanius* (red tailed monkey) are widely distributed and regarded as pests in most agricultural zones and villages in Kenya. In urban centres such as Mombasa and Kisumu, AGMs and blue monkeys are mostly found in city parks and on the forested fringes of the towns. NHPs living in close proximity to human settlements are regarded as potential sources of emerging zoonotic IDs. Recently, a cryptic species of *Oesophagostomum* infecting both NHPs and humans living in sympatry has been described in Uganda suggesting potential infection concerns beyond its accepted foci of infection in Togo and Ghana. This underscores the importance of local scale research for zoonosis risk and epidemiology of this oesophagostomosis. While studies on helminths infecting NHPs in Kenya have focused on captive, rural and animal reserve settings oesophagostomosis, our knowledge on the role of these animals as reservoirs of STH infections in urban settings remains poor. Consequently, determination of nematode's infections occurring in NHPs in close proximity with humans is important because of the following reasons. First, cases of reinfection from NHPs sources hugely compromise drug administration as a control approach, limiting one of the most effective and important tools for control. Second, knowledge on the role of NHPs in transmission is essential in adopting appropriate sanitation approaches that will prevent non-human derived re (infections). Finally, information on the human infective nematodes found in NHPs and their transmission dynamics will be useful in optimising control strategies to break the transmission cycle in densely populated urban centres. Together, insight gathered will be important in informing on appropriate application and effectiveness of current control strategies especially for eradication and elimination target set.

1.6 Scope of the study

This study focused on NHPs found in urban centres of Mombasa and Kisumu, and peri-urban centres within Murang'a and Kakamega counties of Kenya. Previous studies have reported these areas to have high prevalence of STHs in human population. However, data on infection in NHPs that are frequently interacting with human in these regions is lacking despite their eminent potential as reservoirs for helminths. In addition, studies on helminths infecting NHPs have focused on NHPs within wildlife reserves and rural forest habitats leaving information gap on helminth zoonoses originating from free-ranging NHPs within urban and peri-urban centres. Animal sampling was opportunistic. Free-ranging NHPs caught were targeted for translocation to wildlife reserves because they were a menace to the public in urban centres

or were regarded as pests by small-scale farmers within peri-urban areas. Faecal samples were collected from the rectum via swabbing to control for the probability of collecting multiple samples from the same animal for accuracy of infections determined. Species diversity of nodular worm, genus *Oesophagostomum* was determined. While it is considered endemic to west Africa countries, Ghana and Togo, zoonotic cryptic species have been identified in Uganda. Additionally, *O. stephanostomum*, considered a parasite of great apes has been reported in humans. There is therefore a need for local scale research for zoonosis risk and epidemiology of this oesophagostomosis and the role of NHPs as potential reservoirs of the infection in Eastern Africa. This study adds to the limited data on nematodes infections in free-ranging NHPs populations withing Kenya's urban centres.

CHAPTER TWO
LITERATURE REVIEW

2.1. Infectious Diseases (IDs)

Infectious diseases (IDs), disorders caused by pathogenic microorganisms such as bacteria, protozoa, fungi and viruses, have a significant burden on public health and economic stability. They disproportionately affect the poorest populations in the world, most of who live in the tropics (Morens & Fauci, 2013). About 15 million (>25%) of 57 million annual deaths worldwide, are estimated to be related directly to IDs (Morens & Fauci, 2013). Although some, such as smallpox have been eradicated, many persist. In addition, new IDs are emerging (emerging IDs, EIDs) and old ones thought to be under control are recurring (re-emerging IDs, re-EIDs). Notably, some of the IDs are disproportionately common in the tropics, and are associated with poverty hence termed neglected tropical diseases (NTDs). In sum, IDs, whether EIDs, re-IDs or NTDs are of health and economic importance due to huge mortality and morbidity they cause.

2.1.1. Emerging and Re-Emerging Infectious Diseases

EIDs are diseases of infectious origin that have recently appeared within a population, and whose incidence in humans threatens to increase in the near future (Oaks *et al*., 1992; Van, 2014). They include acquired immunodeficiency syndrome (AID) caused by human immunodeficiency virus (HIV), severe acute respiratory syndrome (SARS) caused by a coronavirus, SARS coronavirus (SARS-CoV) and middle east respiratory syndrome (MERS) caused by a corona virus, (MERS-CoV). Ebola virus disease (EVD) caused by Ebola virus, chikungunya caused by chikungunya virus (CHIKV), avian influenza virus (AIV) caused by Avian influenza type A virus and most recently, Zika virus infection caused by Zika virus are examples of EIDs. On the other hand, re-EIDs are IDs that reappear, usually in more pathogenic form and in rapidly increasing incidence or new geographic locations after apparent control or eradication (Racaniello, 2004; Stark & Morgan, 2015) and include cholera, dengue virus (DENV) fever and West Nile virus (WNV) fever. Majority, (60.3%) of these EIDs events result from pathogens (Woolhouse & Gowtage-Sequeria, 2005) with 71.8% of these zoonotic EID events attributed to pathogens with a wildlife origin e.g. Ebola, Nipah virus, HIV and severe acute respiratory syndrome (SARS) virus (Taylor *et al*., 2001; Morens *et al*., 2004). The number of EID events caused by pathogens of wildlife origin has increased significantly with time, and constituted 52.0% of EID events in the recent decades (Jones *et al*., 2018). Rarely identified res-

ervoir hosts maintain many of the human re-EIDs (Liese *et al.,* 2010). Notably, wildlife zoonotic EIDs represent the most significant, growing threat to global health of all EIDs. There is therefore a critical need for health monitoring and identification of new, potentially zoonotic pathogens in wildlife populations making wild animals central in human health.

The most salient example of an EID is HIV/AIDS. It emerged after multiple independent events in which the virus originating from NHPs spread readily within human population after a complex array of social and demographic factors (Morens & Fauci, 2013). HIV came from a central African chimpanzee (*Pan troglodytes*), which harbour a related simian immunodeficiency virus (SIVcpz) (Santiago *et al.*, 2002). HIV-2 originated from the SIVsm of the sooty mangabey (*Cercocebus atys*) monkeys of coastal West Africa, Senegal to the Ivory Coast, the endemic epicentre of HIV-28(Gao *et al*., 1992; Etienne *et al.*, 2011). It has a DNA homology of 40-60% with HIV-1(Etienne *et al*., 2011). Keeping of NHPs as pets and slaughtering them for food in these areas, are suggested routes of transmission to humans, an observation derived from phylogenetic data that imply cross-species infection (Hahn *et al*., 2005). In 2010, the global deaths from human HIV/AIDS were 1.5 million. In 2015, an estimated 35 million people were living with HIV worldwide. Sub Saharan Africa accounts for more than 70% of the global burden of HIV infection (Tanser *et al.,* 2013). High rate of HIV-related sickness and premature adult deaths compromise household stability, diminish labour supply and productivity while increasing costs for households. High active antiretroviral therapy (HAART) is one of the most successful public health interventions on HIV/AIDS. A combination of HIV prevention packages: programs for behaviour change, HIV testing and knowledge of HIV status, abstinence and provision of post exposure prophylaxis, exists. Together, they have transformed a fatal disease to a chronic manageable condition and resulted in decline in co-morbidities and mortality in HIV patients' and rates of new infections. HIV/AIDS as an EID underscore the importance of NHPs surveillance for novel pathogens as a forecast measure for EIDs.

Bats are considered as the natural reservoirs of viruses causing severe diseases in humans e.g. SARS coronavirus (SARS-CoV), Middle East respiratory syndrome coronavirus (MERS-CoV), Nipah virus (NIV), Hendra virus (HeV), and Marburg viruses (Han *et al*., 2015). Although bats are not in close contact with humans, viruses spill over (transmission from reservoir species that maintain a relatively high pathogen population to sympatric population) from bats to intermediate animal hosts, such as horses, pigs or NHPs, is the most likely source of human infection. SARS-CoV, an often-fatal infectious respiratory disease emerged from

bats and spread into humans primarily by person-to-person transmission (Han *et al*., 2015). In 2003, an epidemic of SARS affected 26 countries, resulting in over 8000 cases (Guan *et al*., 2003). Nipah virus also emerged from bats and caused an epizootic in herds of intensively bred pigs. This served as the animal reservoir from which the virus passed on to humans. Human activity is increasingly overlapping the habitats of bats, thus, bat-borne EIDs viruses will undoubtedly increase. Poorly understood spill over events necessitates more research for purposes of bat-borne EIDs prediction and prevention.

Influenza viruses serve as a good example of re-EIDs. Aquatic birds act as reservoirs of avian influenza viruses (AIV) that infect humans, but are generally poorly transmissible in the human host (Webster & Govorkova, 2014). Infections and transmission of AIVs among nonhuman mammals, particularly pigs, facilitate their subsequent transfer to humans in a form that can be readily transmitted (Webby & Webster, 2001; Khiabanian *et al*., 2009; Ma *et al*., 2009). The 2009 H1N1 pandemic virus emerged in humans from pigs following transfer of a swine influenza virus, comprising of a gene segment of swine, human and avian origin (Guan *et al*., 2003). H5N1 and H3N2, subtypes of type A influenza causes the most widespread influenza epidemics while type B viruses causes regional or sporadic outbreaks. Influenza viruses evolve rapidly, changing their antigenic characteristics, necessitating for vaccine modification each year for effectiveness against currently circulating influenza strain (Papazisi *et al*., 2010). In addition, more than 200 subtypes of influenza A infect most species of birds, pigs, horses, dogs and seals (Parrish *et al*., 2014). Evidence for intermediate mammalian hosts in the emergence of human influenza virus is limited. Therefore, better understanding of the ecological, evolutionary, and molecular mechanisms of influenza emergence is necessary for accurate determination of viruses posing a risk to human health. Two distantly related flaviviruses, dengue virus (DENV) and West Nile virus (WNV), are important global health threats re-EIDs. An estimated 400 million DENV infections per year occur, with 3.9 billion people in 128 countries at risk of infection (Brady *et al.*, 2012; Vu *et al*., 2017). WNV is one of the most important arboviral causes of encephalitis worldwide (May *et al*., 2011). Birds are the natural reservoir hosts. Mosquito-bird-mosquito zoonotic transmission cycle primarily involving *Culex* sp., mosquitoes maintain WNV in nature. In Kenya, overall burden of DENV and WNV infection remains largely unknown due to lack of routine arboviral surveillance programs hampering the ability to detect and respond to outbreaks. Epidemic DENV infection outbreak recently occurred in 2013 in Mombasa (Ellis *et al.*, 2015). However, data on human WNV infections in Kenya are sparse, which supports the need for greater surveillance of emerging infections.

Cholera, caused by the bacterium *Vibrio cholerae is* one of the oldest IDs, has repeatedly re-emerged over more than two centuries, and epidemics occur almost every year in Africa. Limited access to clean drinking water and poor toilet facilities are responsible for the epidemics. In addition, overpopulation, poverty, lack of hygiene, poor sanitation facilities, contamination of food and drinking water, risk factors for cholera are endemic in Africa. Between 2000 and 2015, 83% (52812/63658) cholera deaths reported by WHO occurred in SSA (Oguttu *et al*., 2017). Spurred by large outbreak in Tanzania, Haiti, Somalia, Zambia and Burundi in the past decade and recently after cyclone Idai in southern Africa countries, especially Mozambique, the WHO led global task force on cholera control has laid out a roadmap for ending cholera as a public health threat by 2030 (Lessler *et al*., 2018). Access to safe water, appropriate sanitation, and hygiene (WASH) remains the foundation of sustained cholera control despite the fact that the new generation of easy-to-use oral cholera vaccines (OCVs) for prevention of cholera for at least 3 years after administration are available (Bi *et al*., 2017). These vaccines are safe and can curb transmission in the short term, preventing death and disease while making crucial improvements to infrastructure. However, broad use of a vaccine with only transient protection is unlikely to be cost-effective in populations in which few are at high risk, and high population turnover in vulnerable populations might limit its long-term effect. Thus, there is a need to integrate strategies for efficient use of COVs and WASH.

Overall, new human diseases keep emerging. The exponential human population growth globally has led to increased crowding of wildlife and domestic animals, urbanisation and deforestation that alter the habitats of disease carrying insects and animals, all of which increases our risk of exposure to emerging or re-emerging infectious agents. Such EIDs and re-EIDs causes suffering and mortality to a wide population in developing countries in general, and in Africa in particular (Buliva *et al*., 2017). The IDs burden of diseases are beyond mortality, measured in terms of social-economic costs and the health impact measured by disabilities, deformities, loss of productivity, care, and treatment due to infections.

EIDs and re-EIDs represent an increasing and very significant threat to global health and underscore the importance of understanding the factors that increase contact between wildlife and humans and the pathogens they harbour for purposes of developing predictive approaches to disease emergence. Thus, knowledge of the pathogens infecting the free-ranging NHPs is essential in adopting appropriate approaches that will prevent non-human derived (re) infections.

2.1.2. Neglected Tropical Diseases (NTDs)

In December 2003 at a meeting in Berlin on public-health needs of neglected populations, the WHO coined the term "neglected tropical diseases" (NTDs) to describe known diseases that results in a few deaths but impair health and productivity, and are worsened by poverty. The meaning of the term has evolved, and as the name suggests, it describes a group of communicable diseases that occur in tropical and subtropical regions, that have received limited global and financial attention as compared to malaria, HIV/AIDS and tuberculosis (TB). Also called "diseases of the poor or poverty", NTDs are not restricted to the tropics, but occur in areas of extreme poverty, including arctic regions and North America and Europe (Hotez *et al*., 2008; Hotez, 2010) (Figure 1).These diseases, which are more than 17 in number, occur in 149 countries with over one billion people with one or more infections and approximately 4 billion at risk of infection (Lustigman *et al*., 2012). They include bacterial (e.g. Buruli ulcer and leprosy), viral (dengue and chikungunya), protozoan (sleeping sickness and leishmaniasis) and helminths (soil-transmitted helminths and cysticercosis) infections among others. In 2005, WHO expanded the NTDs list to include among the helminth infections, echinococcosis and foodborne trematodiases, Chagas disease to the list of protozoan infections, while yaws (endemic treponematoses) was added to bacterial infections. Two arbovirus infections–dengue and chikungunya, as well as rabies were added: viral infections were not on the original list. A group of fungal deep mycoses was also added in addition to snake bite envenomation (Hotez *et al*., 2020).

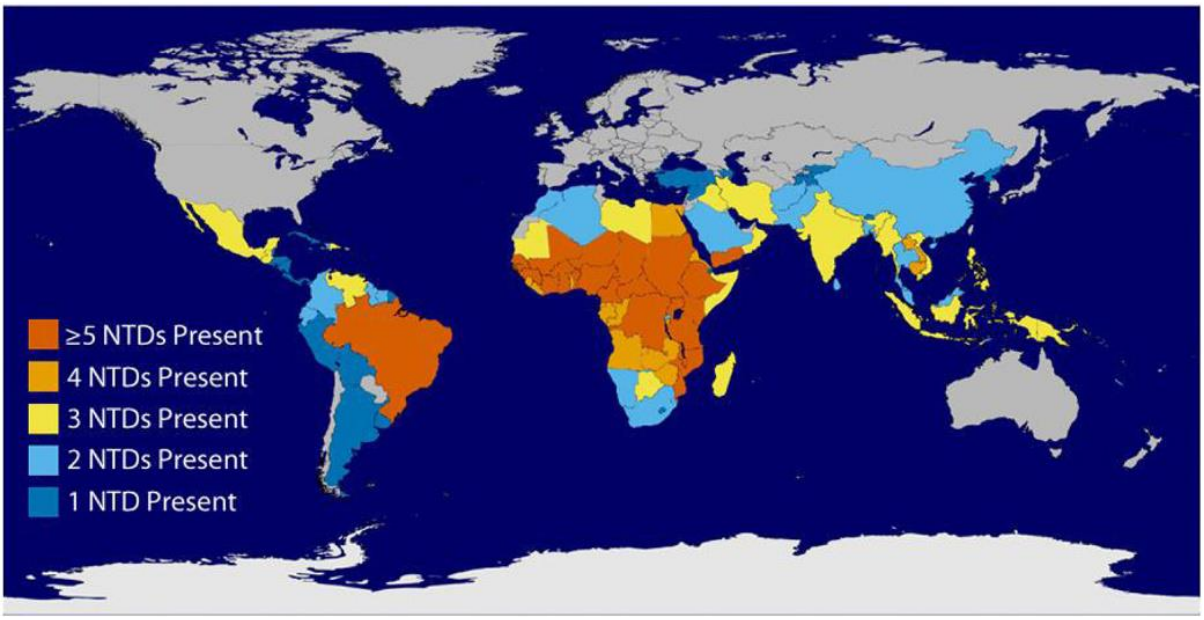

Figure 1: Global overlap of the six most common neglected tropical diseases (NTDs), Bhutta *et al*. (2014). NTDs occur principally in tropical and subtropical regions but are also found in arctic regions, North America, and Europe. Co-infections are common, and can involve more than five diseases as shown in the colour codes inset. The poorest regions of the world which are in the tropics and include Africa, South America and Asia have the highest burden. Figure adapted from Bhutta *et al*. (2014).

Buruli ulcer is a necrotising disease of skin and bone caused by *Mycobacterium ulcerans* and usually occur near rural tropical wetlands. An estimated 7000 cases of Buruli ulcer are reported annually worldwide (Walsh *et al*., 2008) with more than 4000 of these occurring in SSA. In spite of considerable research efforts during the past few years, transmission and environmental reservoirs of *M. ulcerans* remain elusive (Roltgen *et al*., 2010). However, risk factors include proximity to slow flowing water and age (<15 years) (Walsh *et al*., 2008). This serious NTD remains a major health problem in many parts of the world, but in particular, in West and Central Africa. Current control strategies rely on active case search in regions suspected to be endemic and subsequent antibiotic treatment using a combination of rifampicin and streptomycin. Therefore, there is a need to direct research in the identification of animal reservoirs for *M. ulcerans* in Africa to provide effective prevention strategies.

Leprosy is a chronic granulomatous infection caused by *Mycobacterium leprae* an obligate intracellular organism. It mainly affects the skin and peripheral nerves leading to severe physical disabilities and deformities if not diagnosed and appropriately treated. Standard treatment is multi-drug therapy, consisting of the combined administration of three antibiotics namely, rifampicin, clofazimine and dapsone, or two, rifampicin and dapsone, depending on

the bacillary load. *Mycobacterium leprae* transmission is primarily person-to-person, through prolonged close contact between susceptible and genetically predisposed individuals and untreated multibacillary patients by inhalation of bacilli present in the upper airway secretions. The WHO target to eliminate leprosy as a public health problem has significantly increased worldwide access to treatment, thereby remarkably reducing the prevalence of the disease. However, there are 192,246 cases of global burden due to leprosy, with 226,474 new cases in 105 countries detected in 2010 (Rodrigues & Lockwood, 2011). In 2017, the registered leprosy prevalence was 193,069 cases with 210,973 new cases detected (Smith *et al*., 2017). Thus, active transmission still occurs, despite the availability of treatment. Human beings are the reservoirs of *M. leprae* (Lastoria *et al*., 2014), but animals, such as armadillos, chimpanzee and other apes act as reservoirs. In addition, the pathogen occurs in soil, water and some arthropods, which could be playing a role in the continued leprosy transmission.

Human African trypanosomiasis (HAT) and leishmaniasis are major kinetoplastid diseases in SSA and accounts for almost 2 million Disability Adjusted Life Years (DALYs) lost annually (Gilbert *et al*., 2016; Mitra & Mawson, 2017). HAT in SSA occurs within the distributional limits of the vector, the tsetse fly (Simarro *et al*., 2010). Two forms of the disease exist: a slow progressing form, caused by *Trypanosoma brucei gambiense* is endemic in western and central Africa (Gambian HAT), and, the faster-progressing form, caused by *Trypanosoma brucei rhodesiense* endemic in eastern and southern Africa (Rhodesian HAT). The latter causes approximately 98% of the cases (Buscher *et al*., 2017), while *T. b. rhodesiense* is responsible for the remaining cases in SSA. Of the 2,804 HAT cases reported in 2015, *Trypanosoma brucei gambiense* caused 2,733 while *Trypanosoma brucei rhodesiense* caused 71 cases (WHO, 2017). In the absence of vaccine, disease control relies on detection and treatment, and to a lesser extent, vector control. The drugs used include pentamidine, suramin, melarsoprol and eflornithine. However, they have a major disadvantage that limits their widespread use in the endemic regions of SSA. Melarsoprol for instance is toxic and has increasing treatment failures while eflornithine is expensive, laborious to administer, and lacks efficiency against *T. b. rhodesiense. Trypanosoma brucei gambiense* resistance to melarsoprol and pentamidine have been documented (Babokhov *et al*., 2013). Recently, competent, safe, combination therapy of nifurtimox-eflrnithine combination treatment (NECT) is available (Buscher *et al*., 2017). Additionally, diagnosis and treatment are resource intensive activities and require specific training, which is difficult to ensure in all countries and endemic areas.

A vector, phlebotomine sand flies, transmits leishmaniasis. It has three distinct clinical syndromes, cutaneous leishmaniasis and mucocutaneous leishmaniasis which affects the skin,

and mucous membranes respectively and, visceral leishmaniasis (VL) which affects internal organs. Both visceral and cutaneous forms occur in SSA, with the former producing a serious disease associated with high mortality. The Pokot territory of Kenya and Uganda is endemic (Kolaczinski *et al*., 2008). In these areas, VL is primarily an anthropotonic infection (Kolaczinski *et al*., 2008). Cutaneous leishmaniasis caused by infection with *Leishmania major* is also endemic in parts of SSA, including West Africa (Bern *et al*., 2008). There is no vaccine for VL. Chemotherapy involves treatment using pentamidine, paramomycin, miltefosine, liposomal amphotericin B and pentavalent antimonial. Strategies employed for reducing transmission risks are mainly control of vector populations and reservoirs and by reduction of contact with infected sand flies by using long lasting insecticide treated bed net.

Trachoma is a leading cause of infectious and preventable blindness worldwide (Taylor *et al.*, 2014). Active trachoma affects an estimated 21 million people globally, with about 2.2 million severely visually impaired or blind with a further 7.3 million having trichiasis. Of the 21 million cases of active trachoma globally, 48% occur in SSA. Trachoma distribution is mainly in savannah areas of East and Central Africa and the Sahel of West Africa (Reddy *et al*., 2007). Like other NTDs, a setting of poverty, poor facial hygiene and young children who act as reservoirs of infection sustains trachoma (Wright *et al*., 2008). Dry zones with limited water accessibility and hot lowlands (altitude < 3000 m) with dense fly populations, promote transmission (Koloczinski *et al*., 2008). Control involves administration of an oral drug, azithromycin, in MDA to treat the ocular strains of chlamydia that cause the blinding disease (Taylor *et al*., 2014), surgery to correct the upper eyelid deformity and facial cleanliness. Muscid flies are important vectors of trachoma and may be useful for reducing trachoma. While trachoma, leishmaniasis, Human African Trypanosomiasis, buruli ulcer and leprosy are NTDs they are not as a result of soil transmitted helminth infection but serve to demonstrate importance of wild reservoirs in disease transmission dynamics.

Preventive chemotherapy and transmission control NTDs (PCT-NTDs) are a subset of NTDs that can be controlled or eliminated through MDA in eligible populations without prior individual diagnosis (WHO, 2017). They include lymphatic filariasis (LF), onchocerciasis, schistosomiasis and the soil transmitted helminth infections (STHs) such as roundworm, hookworm and whipworms, which account for more disability- adjusted life years (DALYs) than better known conditions such as malaria (Hawdon,2014). Their public health burden is huge, causing substantial disability ranging from skin and mucous membrane irritation to disabling lymphedema and hydrocele, organ damage and growth and cognitive deficits. They also contribute to large-scale lost productivity, stigma and discrimination especially in SSA where they

are endemic (Hotez *et al.*, 2009). In light of rapid urbanisation, reaching at high-risk populations in urban centres is a priority for NTD programs globally, especially in cases of LF and STHs infections, as well as onchocerciasis and schistosomiasis in peri-urban settings (Adams *et al*., 2018). In addition, increased contact with NHPs challenges the effectiveness of MDA programs. As highlighted in a 2010 survey of African NTD program managers, urban specific guidance from the WHO organisation on mapping and treating in urban areas is lacking (Adams *et al.*, 2018). Therefore, concerns about the amplification of several STHs are valid, more so if achieving the WHO 2020 target of elimination and eradication of STHs are to be met. Identification of reservoirs, especially the role that NHPs may play in the continued transmission of STHs is thus important.

2.2. Helminths and Helminthiases Impact

Helminths or worms are a diverse group of non-parasitic (annelids) and parasitic (nematodes and platyhelminths) metazoan organisms. Helminthiases are caused by nematodes (roundworm), trematodes (flukes) and cestodes (tapeworms), part of NTDs of major public health concern, and have been so since ancient periods (Hotez *et al.*, 2006); helminth infections are described in ancient writings of Hippocrates, Egyptian medical papyri and even the Bible (Cox, 2002; Hotez *et al.*, 2006). Notably, 85% of the NTDs disease burden results from helminth infections (Bhuttah *et al*., 2014). Globally, about 1.3 -2 billion people are infected (Hawdon, 2014) with more at risk. Of concern are the STH infections, the most common NTDs worldwide. They disproportionately affect the poor (Gangly *et al.*, 2017) and are widely distributed in tropical and subtropical areas (Figure 2). They constitute serious public health concern, especially in developing and underdeveloped countries where sanitation is poor; poverty is endemic and tropical climate that supports their life cycle. Most of these infections affect children and young adults with tremendous health and development impacts. Further, infections of severe intensity can impair physical growth and cognitive development. They are also a cause of micronutrients deficiencies leading to poor school performance and absenteeism in children, and reduced work productivity (Hotez & Kamath, 2009; Liese *et al*., 2010). An estimate of their disease burden in DALYs in SSA is 5.4-18.3 million compared to 9.3 million DALYs for malaria (Tabi *et al*., 2018). In Kenya, STH infection prevalence is highest at the coastal, central and western Kenya regions, with more than 10 million people being infected and approximately 16.6 million people being at risk (Mwandawiro *et al*., 2019). Majority of those infected are asymptomatic or suffer minor symptoms compared to other bacterial or viral infections while a small percentage suffer severe life-threatening consequences.

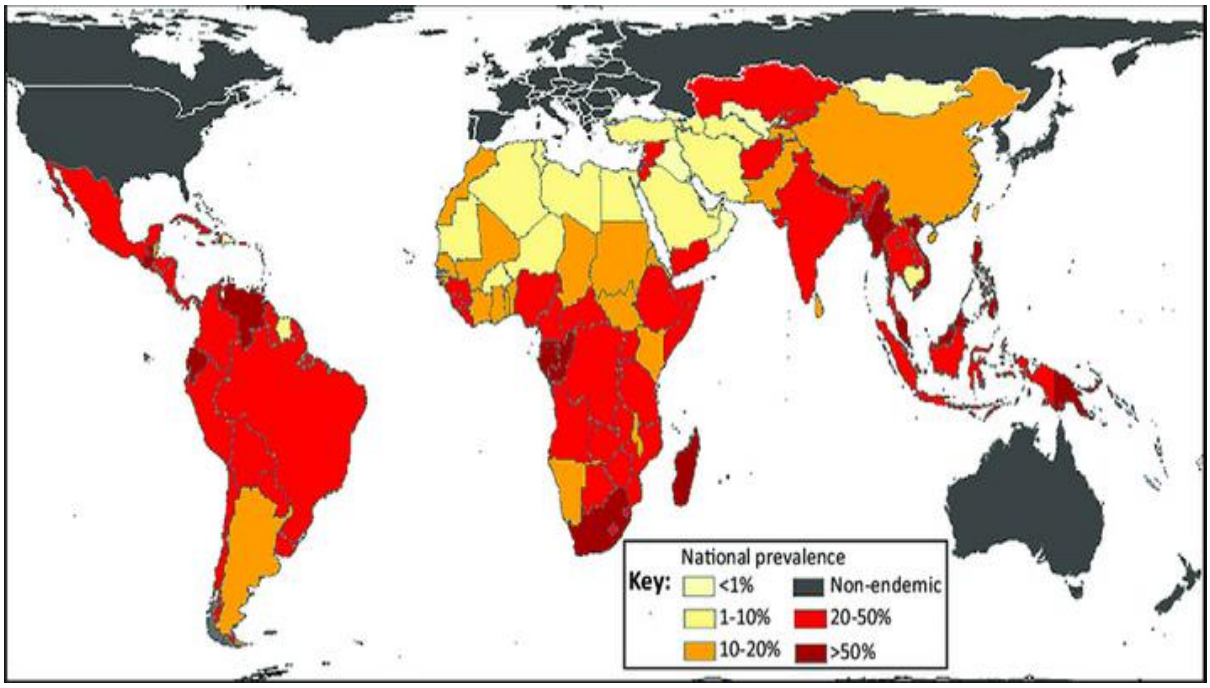

Figure 2: Global distribution of soil transmitted helminths (STHs). A map showing global estimates of STHs namely *Ascaris lumbricoides, Trichuris trichiura, Necator americanus* and *Ancylostoma duodenale,* which are endemic in tropical and subtropical regions. The key show prevalence in percentage. The greatest prevalence, 20-50% and >50% occur in sub-Saharan Africa, Asia and South America causing an estimated disease burden of more than 5 million DALYs. Figure adapted from Campbell *et al.* (2016).

Globally, over one billion people are infected by at least one of the commonest species of STH namely *Ascaris lumbricoides* (the roundworm), *Trichuris trichiura* (the whipworm), *Strongyloides stercoralis* (threadworm) and the hookworms: *Ancylostoma duodenale* and *Necator americanus* (WHO, 2015) of which 300 million suffer associated severe morbidity and even death. An estimated 173 million and 163 million people in SSA suffer infections with *Ascaris* sp., and *Trichuris* sp., respectively, with 36 million school-aged children infected with Ascariasis and 44 million with Trichuriasis. High prevalence rates of Ascariasis and Trichuriasis are often present in Africa's urban areas compared to rural areas, unlike hookworm, which is more evenly distributed.

Strongyloidiasis causes diarrhoea and malnutrition in SSA, although there is little information on its distribution or disease burden, in part because of the difficulties in diagnosing this infection; use of conventional techniques fail to detect larvae in up to 70% of cases. WHO recommends annual treatment in areas where the prevalence rate of STH is between 20% and

50% and bi-annual treatment in areas with prevalence rates over 50%. With such huge impact, there is a critical appraisal of control strategies for STHs.

2.3. Helminth Transmission routes

There are various transmission routes for helminths. They include faecal-oral and geophagy and transdermal routes (Figure 3).

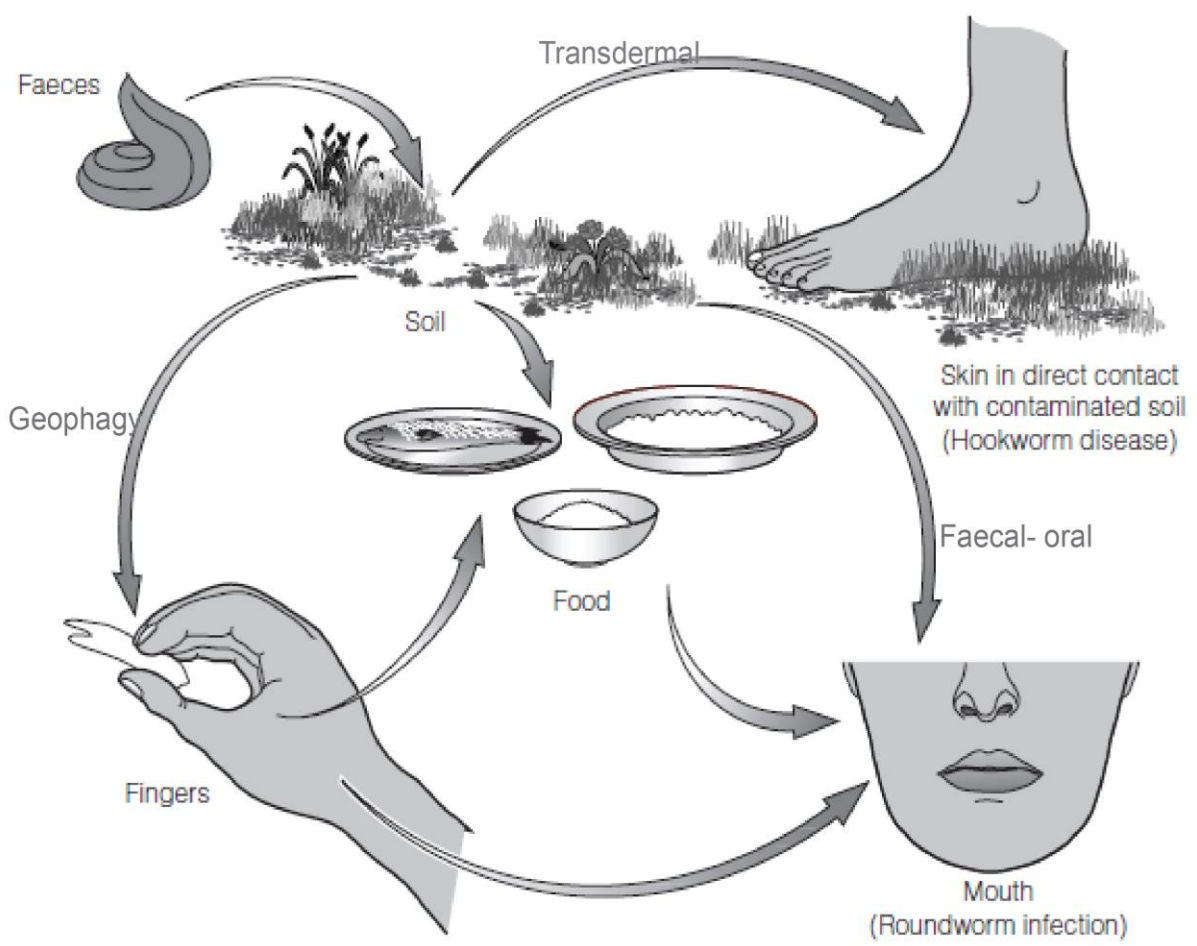

Figure 3: The STHs transmission routes. The helminth eggs and/or larvae are shed into the environment in host faeces. They can be contracted through ingestion of contaminated food and/or water (faecal-oral route) and soil eating (geophagy). In some species, eggs shed in host faeces develop into infective larvae while in some, larvae are shed in the feces, these can be contracted through the skin (transdermal route). Image adapted from Rottier and Margaret, 2003.

2.3.1. Faecal-oral and Geophagy Transmission

The most common route of infection, especially in the developing countries is faecal-oral route (Figure 3) through internalisation of infected foodstuff (Mohamed *et al.*, 2016). Geophagy or dirt eating, especially by pregnant women due to deficiency of some minerals in their diet is another route of infection in SSA (Johnson *et al.*, 2010). Areas that neighbour forests have a high risk of soil contamination from NHPs faecal waste or sharing watering points with humans, leading to infection and /or re-infection even after treatment. This necessitates understanding of the distribution of human infective helminths that NHPs harbour in order to optimise control strategies. Understanding of the potential reservoirs and sources of infection is important in efforts to eliminate and eradicate helminths as it provides insights useful in disruption of transmission.

2.3.2. Transdermal Transmission

Walking barefoot on soils contaminated with human or NHPs faecal matter might lead to the penetration of infective larvae through the skin (Figure 3). Exposure to soils where infective stages of hookworm inhabit, more so in cases of poor hygiene, particularly defecation practices that lead to shedding of helminths into the environment are risk factors for helminth transdermal infections (Olsen *et al.*, 2001). Such contamination can also result from faecal matter from NHPs, some of which act as reservoirs of these pathogens hence crucial players in transmission dynamics. Our accurate knowledge of their role is important in ensuring appropriate deployment of various control strategies.

2.4. Life Cycle, Pathology and Clinical Symptoms of STHs

Understanding the life cycle of parasites provides information of epidemiological significance that is indispensable to developing effective control programs. It is also useful as a predictive value with respect to the pathogenic importance of each parasite.

2.4.1. *Trichuris* sp

The genus *Trichuris* contains more than 20 described species that parasitise a range of mammalian species (Dolezalova *et al*., 2015). *Trichuris trichiura,* (whipworm) is considered the third most common roundworm to infect humans with an estimated over 600 million people infected worldwide (Bethony *et al.*, 2006; Bethony *et al*., 2011; Liu *et al*., 2012). Whipworms also infect other animal hosts, including pigs, dogs and NHPs, and causes disease similar to human Trichuriasis. Three *Trichuris* species; *T. suis, T. trichiura and T. vulpis* are zoonotic parasites, and are a threat to human health (Taylor *et al*., 2001). Transmission occurs through

faecal-oral route (see Figure 3). The infective thick-shelled (embryonated) eggs ingested in contaminated foodstuff or in geophagy (soil/dirt eating) hatch after gastric passage in the small intestine releasing first stage larvae (L1s). L1s migrate to the large intestine (caecum and colon), where they develop and moult multiple times into adults (~30–50 mm-in length). The worms burrow their thin thread-like anterior end into the mucosal lining of the large intestinal wall, feed on tissue fluids, mature and produce eggs. See Figure 4. In the large intestines, large number of worms' cause disease (Trichuriasis), which is usually associated with enterotyphlocolitis and clinical signs, such as dysentery, bloody diarrhoea and/or rectal prolapse. Children (~5-15 years of age) often harbour the largest numbers of worms (Hotez, 2009).

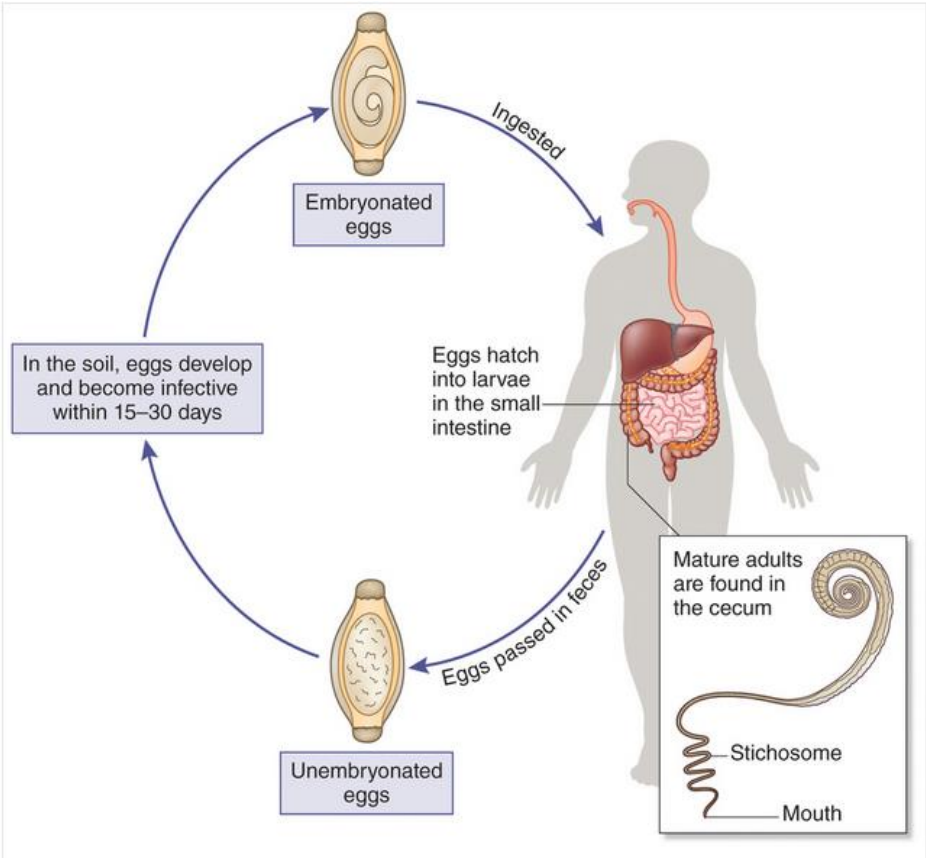

Figure 4: Life cycle of *Trichuris trichiura*. Un-embryonated eggs are passed with stool by infected human or NHPs to soil where, after 15-30 days, they become embryonated and enter the infective stage. Infection occurs upon ingestion of the embryonated eggs in contaminated foodstuff. They hatch in the small intestine to release larvae. The larvae mature in the colon into adult worms. The adult worms live in the caecum and ascending colon, with the anterior portions threaded into the mucosa. The females begin to lay eggs 60-70 days after infection. The eggs are shed into the environment with faeces where under good conditions of temperature and moisture they embryonate. Host ingests the embryonated eggs and the cycle starts afresh. Figure adapted from Ghedin, E. 2014.

Diagnosis is by microscopy, visualisation of the characteristic barrel shaped eggs in the faeces. The eggs measure 50-55 mm by 22-24 mm, are yellowish to dark brown in colour and present "plug like" prominences at each pole (bi-polar plugs) (Stephenson *et al.*, 2001). Albendazole used in treatment of Trichuriasis has low curability, but greatly reduces egg production (Bennet & Guyatt, 2000) which might decrease sensitivity of microscopic examination. Therefore, polymerase chain reaction (PCR) offers better diagnostic alternatives owing to their high sensitivity and specificity.

2.4.2. *Oesophagostomum* sp

Soil transmitted helminths of the genus *Oesophagostomum* termed nodular worms are intestinal nematode parasites of pigs, ruminants and primates. Human cases have been attributed to a zoonotic origin, with NHPs proposed as the potential reservoirs (Lieshout *et al*., 2005). Of the eight NHPs infecting *Oesophagostomum* sp recorded to date, *O. bifurcum, O. stephanostomum and O. aculeatum* are of major importance due to their potential to infect humans (Ghai *et al*., 2014). *Oesophagostomum bifurcum* the principal nodular worm of humans, is highly prevalent and of major human health concern in focally endemic areas of Africa, Ghana and Togo (Ota *et al*., 2015). However, factors explaining such a high regional prevalence remain unknown. While the prevalence and distribution of Oesophagostosomiasis in West Africa has been extensively studied, (Lieshout *et al*., 2005; Arizono *et al*., 2012; Makouloutou *et al.*, 2014) it is unclear to what extent the NHPs in Kenya harbour the *Oesophagostomum* sp and their potential as reservoirs for human infections. Recently, a study reported infection involving a novel *Oesophagostomum* (a phylogenetically over-dispersed previously uncharacterised taxon) in Uganda, East Africa, among primates and sympatric humans (Ghai *et al*., 2014). It thus suggests broad transmission among species of distantly related primate hosts, including humans i.e. zoonotic transmission might be common.

Oesophagostomum transmission is via faecal-oral route (Figure 3). Infection occurs by ingestion of the L3 larvae that hatches under favourable conditions from egg shed in faeces released into the environment by infected NHPs and humans. These penetrate the intestinal wall. Some of these develop rapidly into young adult worms that return to the intestinal lumen to mate and produce eggs while others develop into immature worms and stay in intestinal wall. Eggs produced by adults in the intestinal lumen hatch into stage one larvae (L1), the rhabditiform larvae. The L1 through two moult stages develop into infectious stage three (L3) larvae (filariform larvae) in five to seven days. The larvae are characterised by triangular intestinal cells, long and finely tapered tail of sheath (membrane) and the prominent transverse

striation of the sheath, useful in differentiation from hookworm. Primates, including humans ingest the L3 larvae in contaminated food and water. After ingestion, L3 larvae burrow into the submucosa of the intestines and induce cysts. The larvae moult within cysts, into stage four larvae (L4). L4 larvae migrate back to the lumen of the large intestine, where they moult into adults, mate and produce eggs that appear in faeces of the definitive host about a month after ingestion of infective L3 larvae. They are ovular in shape, thin shelled and range from 50 -100 µm in size. Freshly excreted eggs contain a developing embryo in the early stages of cleavage.

The infection causes abdominal pain, anorexia, diarrhoea and cachexia, and occasionally death from peritonitis and intestinal occlusion (Kumar *et al.*, 2015). Clinical diagnosis is often difficult; common oesophagostomiasis misdiagnosis is carcinoma, appendicitis or amoebiasis laparotomy, a surgical incision into the abdominal cavity, provides a positive diagnosis but it is expensive. Diagnosis by microscopy is unspecific and requires cultivation of eggs to L3 larvae via faecal cultures to differentiate them from hookworm egg (Kumar *et al*., 2009). The WHO recommended treatment is by administration of albendazole, which is effective even on tissue dwelling stages.

2.4.3. *Strongyloides* sp

Nematodes of the genus *Strongyloides* are parasites of vertebrates, with over 60 species described from mammals, birds, amphibians and reptiles (Thompson *et al.*, 2008; Hasegawa *et al.*, 2016). Wild great apes and other non-human old-world primates habour *Strongyloides* infections with a high prevalence (Rothman *et al.*, 2008; Freeman *et al*., 2013; Ota *et al*., 2015). Of these, two species are known to infect humans, *S. stercoralis* and *S. fuelleborni* (Dorris & Blaxter, 2002; Viney & Lok, 2015) although the latter is clinically less important being principally a NHPs parasite and with a restricted geographical distribution in Papua Guinea and Africa. *S. stercoralis* infects an estimated 370 million individuals (Dacal *et al*., 2018).

The life cycle of *Strongyloides* sp is unique among nematode parasites of vertebrates because of its alternation between free-living and parasitic cycles, and its potential for autoinfection and multiplication within the host (Figure 5). Transmission is via skin penetration (transdermal) by infective filariform (L3) larvae (Figure 3). *Strongyloides* adult worm lives in the mucosa and submucosa of the duodenum and jejunum where they release eggs that hatch in the bowel lumen, liberating rhabditiform larvae. Rhabditiform larvae excreted in stool of infected host, including NHPs and humans develop into infective filariform larva. The larvae penetrate host skin in contact with contaminated soil or water, migrate through the bloodstream to the lungs, break through pulmonary capillaries, ascend the bronchial tree to the pharynx, are

swallowed, then reach the small intestine, moulting via a fourth larval stage (L4) into adult parasites (Puthiyakunnon *et al.*, 2014).

In the soil, larvae that do not contact a host develop into free-living adult worms that reproduce for several generations before their larvae re-enter a host. The free-living adults' mate and the female lays eggs that hatch to release stage one larvae (L1) that moult via a second larvae stage (L2) into an infective third stage (L3) larvae, the filariform stage. All the progeny of the free-living adult generation is female. The infective L3 stage persists in the environment for long periods until they encounter a suitable host. In addition, females L1s that hatch from eggs passed in faeces can moult via an L2 into infective L3s. Some rhabditiform larvae convert within the intestines to infective filariform larvae (L3) that immediately re-enter the bowel wall, short-circuiting the life cycle (internal autoinfection). Sometimes filariform larvae passed in stool re-enter through the skin of the buttocks and thighs (external autoinfection). Autoinfection explains why Strongyloidiasis can persist for many decades and helps account for the extremely high worm burden in the hyper-infection syndrome. Autoinfection appears to be unique to *S. stercoralis* but *Enterobious* sp and *Capillaria* sp., also have this phenomenon and largely accounts for their being serious pathogens of humans.

Symptoms of Strongyloidiasis include diarrhoea, nausea, abdominal discomfort, vomiting, dermatitis, pruritus and respiratory tract symptoms such as cough, asthma and dyspnoea (Groove, 1996; Puthiyakunnon *et al*., 2014). Hyper-infection or disseminated Strongyloidiasis can affect several organs, leading to fatal outcomes. Chronic asymptomatic Strongyloidiasis is another significant concern when coupled with immunosuppressive treatment; it has the potential to develop into disseminated infection (Puthiyakunnon *et al.*, 2014).

The larvae, other than eggs are secreted in faeces in *S. stercoralis* while eggs rather than larvae are shed in *S. fuelleborni* infection, especially in children. *Strongyloides* hyper infection can result in high number of larvae in stool samples. The eggs and larvae are easily diagnosed using microscopic techniques. Direct smear examination of stool in saline and Lugol's iodine stain for detection of larvae in stool is a definitive diagnostic test. However, a single direct stool examination alone may be inadequate as the egg output compared to other parasitic helminths is too low. Other methods of diagnosis include Baermann's and formalin-ethyl acetate concentration techniques, which improves sensitivity of stool exams (Requena-mendez *et al.*, 2013). Faecal samples can also be grown in faecal cultures from which infective L3 can be obtained.Thiabendazole, Albendazole and Mebendazole are used for the treatment of acute and chronic Strongyloidiasis.

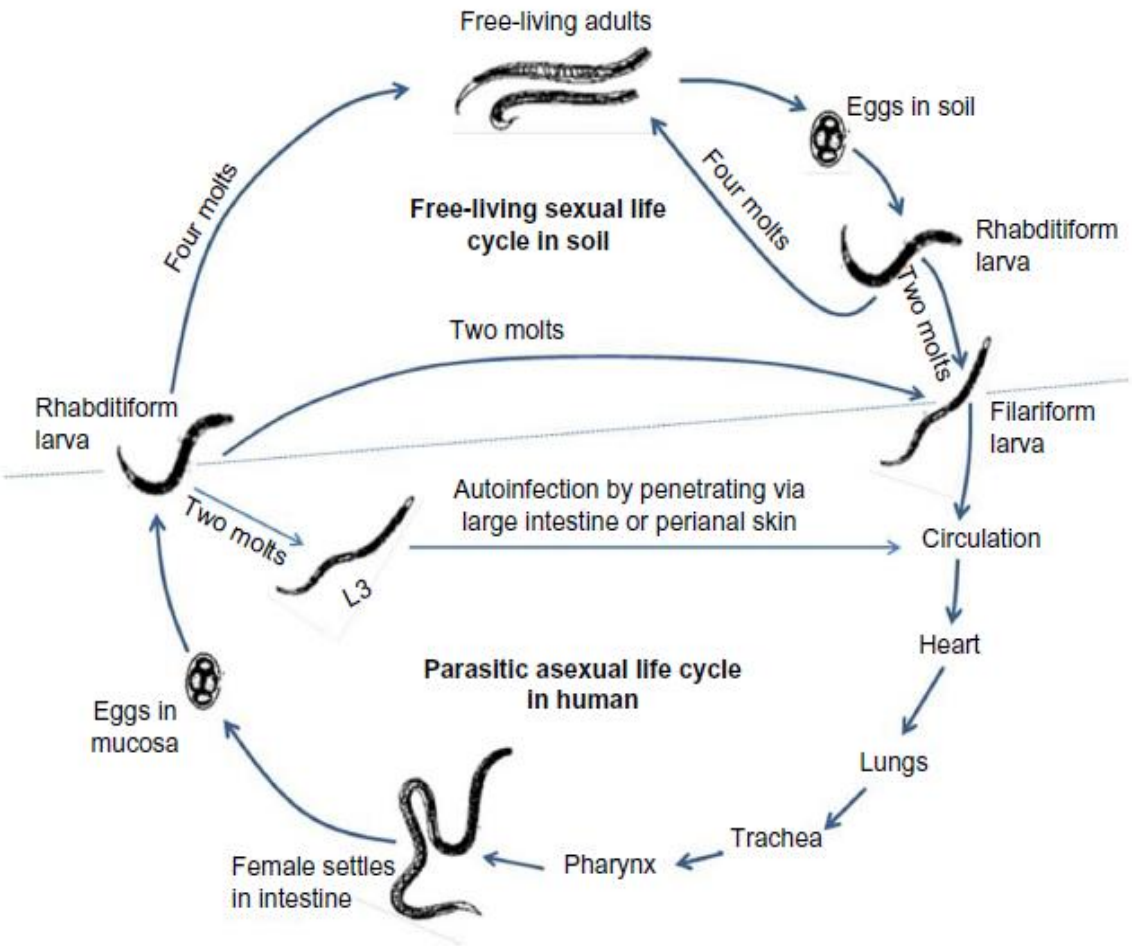

Figure 5: The life cycle of *Strongyloides stercoralis.* The rhabditiform larvae passed in the stool of infected non-human primates (NHPs) or humans may develop into infective filariform larvae (direct) or a free-living adult male or female worm. The free-living forms mate and produce eggs that hatch into rhabditiform larvae and eventually into filariform larvae. The filariform larvae is the infective stage that penetrates host skin in contact with contaminated soil or water, migrate through the bloodstream to the lungs, break through pulmonary capillaries, ascend the bronchial tree to the pharynx, are swallowed, then reach the small intestine, moulting via a fourth larval stage (L4) into adult parasite. Adult worms live in the mucosa and sub mucosa of the duodenum and jejunum where they release eggs that hatch in the bowel lumen, liberating rhabditiform larvae that are excreted in stool. Some rhabditiform larvae convert within the intestine to infective L3 that immediately re-enter the bowel wall, short-circuiting the life cycle (autoinfection). Figure adapted from Ravi and Kakuturu, 2016.

2.4.4. *Ascaris lumbricoides*

Ascariasis is a disease caused by the nematodes *Ascaris lumbricoides*, the largest gastrointestinal roundworm, and giant roundworm. It is zoonotic, affecting both humans and

NHPs. An estimated 800 million to 1.2 billion people are infected worldwide and causes more than 60,000 deaths annually (Shah & Shahidullah, 2018). Infections are endemic, but not restricted to the tropical and subtropical areas (Chan *et al*., 1994; Crompton, 1999) as they also occur in Europe and in Austria, where it is the third most prevalent helminthic infection (Tomaso *et al*., 2001). Their life cycle requires no intermediate host, the definitive host is human, and the mode of transmission is the faecal-oral through ingestion of embryonated eggs (see Figure 3 and 6). The eggs are released in faeces of infected humans and NHPs. Under favourable conditions (warm, shady and moist), fertilised eggs embryonate and become infective. Following ingestion, the embryonated eggs hatch into rhabditiform larvae that penetrates the wall of the duodenum and enters the portal venous system and lymphatic channels, where they migrate hematogenously and via lymphatics to the liver and then the lungs. In the lungs, the larvae move into the alveoli where they mature over a period of 10-14 days before they make their way up the tracheobronchial tree to the hypopharynx where they are swallowed. On returning to the small intestine, they mature into adult worms that live in the lumen, typically of the jejunum or ileum. When both female and male adult worms coexist, they mate and the females produce fertilised eggs that are passed into the stool. The fertilised egg is broadly oval, with a thick shell with an outer, course, mammilated albuminous covering. It measures 45 to 75µm in length by 35 to 50µm in breadth, and are stained brown from bile. Under warm, shady and moist conditions, the eggs can remain viable in soil for up to 10 to 15 days' time in soil to become infective. Arrival of larvae back to the small intestine concludes the extra intestinal migration of larvae and begins another moulting to become adult worms (De Lima Corvino *et al*., 2018).

Pathogenesis of ascariasis is generally related to organ damage and host reactions to larval migration as well as the number and location of adult worm in the body. *Ascaris* larvae migrating through the intestinal mucosa, liver and lungs provoke hypersensitivity reaction in the human host. Some of the larvae may be immobilised and covered with eosinophils, resulting in the formation of granulomas. In the lungs, movement of the larvae from the blood vessels into the air spaces results in haemorrhage. There is oedema of the alveoli. Alveolar sacs are filled with a serous exudate, the peri bronchial tissues becoming infiltrated with eosinophils and neutrophils, and mucus production in the bronchi is increased. Known as Loeffler's syndrome, it gives rise to dry cough, high fever and bronchial asthma. The effect is severe when the numbers of larvae is large or when transmission is seasonal.

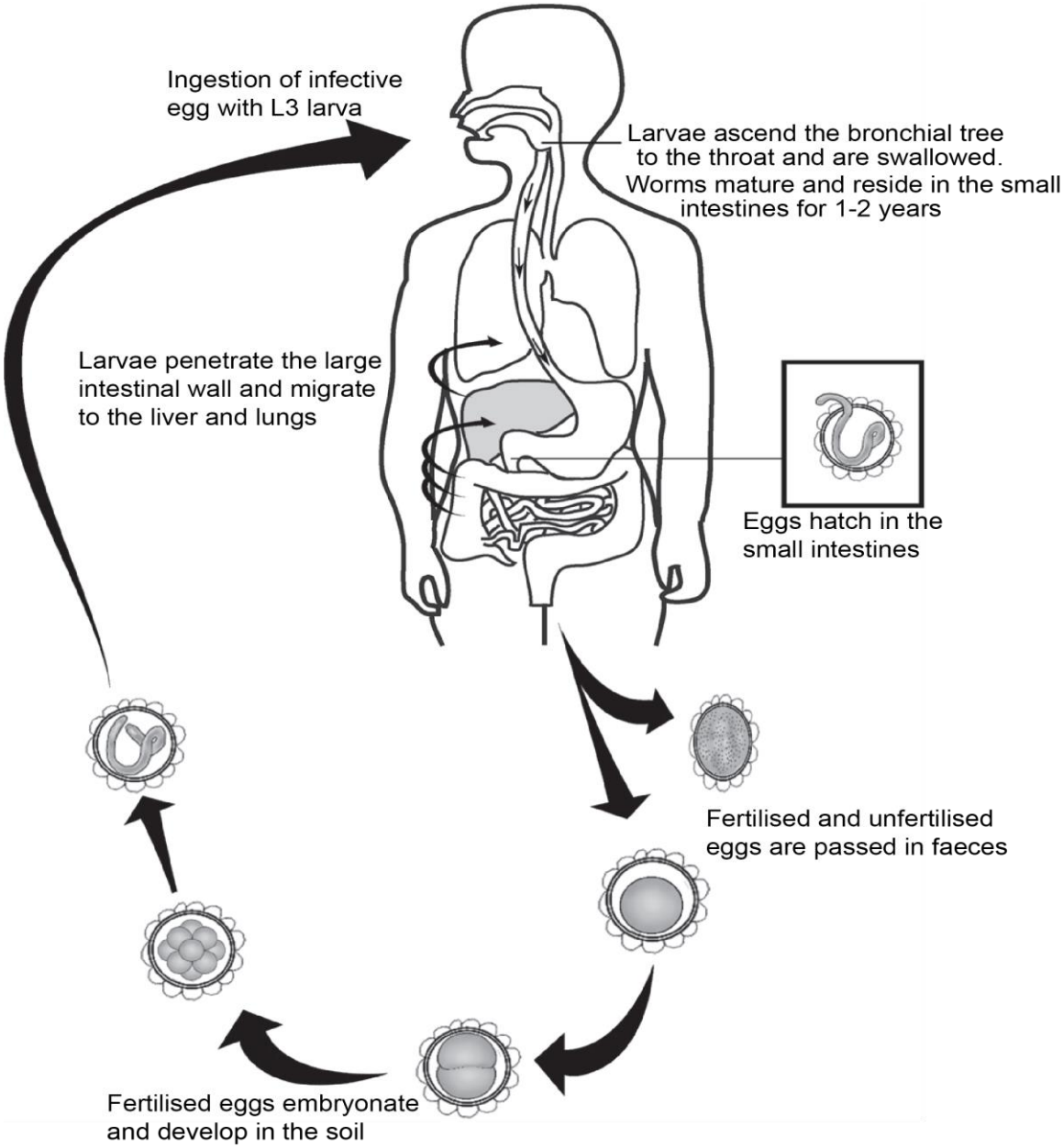

Figure 6: Life cycle of *Ascaris lumbricoides.* The eggs, both fertilised and unfertilised are released in faeces by the female adult worm residing in the small intestine lumen of infected host. Only fertilised eggs are infective upon ingestion. They require moisture, warmth, shaded soils to embryonate and become infective. This takes 18 days to several weeks. Upon ingestion, the infective eggs hatch into larvae, invade the intestinal mucosa, and are carried via the portal, then systemic circulation to the lungs. After about 14 days in the lungs the larvae mature and penetrates the alveolar walls. They then ascend the bronchial tree to the throat from where through epiglottis they re-enter the gastrointestinal tract to the small intestine. They mature into adult worms in the small intestine and can live up to 2 years. Fertilisation occurs in the small intestine. Figure adapted from Dold and Holland, 2014.

For diagnosis, faecal examination using direct smear or concentration techniques for ova can be done because the adult female produces as many as 200,000 eggs per day (Murray *et al*., 2005). However, previous reports have suggested egg production is reduced after treatment, hence might give rise to false negative when using microscopic examination (Roberts & Janovy, 2009). The WHO recommended drug for treatment, levamisole, is highly effective and well tolerated. It acts on the worms' nerve ganglia paralysing the musculature within minutes of contact resulting in the immediate ejection of the worms by normal peristaltic movement in less than 24 hours. Levamisole is available as tablets and drinkable suspensions.

2.5. Diagnosis of Nematode Infection

Accurate species identification is important in designing and application of effective nematode management practices. Various techniques, invasive or invasive are used for diagnoses of nematode infections. They include, direct smear, kato-katz, coproculture and concentration techniques, sedimentation and floatation.

2.5.1. Direct Smears

Direct thin smear involves examination of a thin smear of faecal material with normal saline or iodine on a microscope slide. It is relatively easy to perform, has low cost and is time saving. However, it is effective only when concentrations of egg or larvae are high. Low sensitivity of the direct wet mount technique has been reported in the detection of low intensity infections (Levecke *et al*., 2009). In addition, large amounts of detritus in faeces can interfere with identifications and quantitative assessment of egg production is not possible (Periago *et al*., 2015).

2.5.2. Concentration Techniques

Number of parasitic forms of helminthic parasites in faecal samples is often too low to for microscopic observation in direct wet mounts or stained smear preparations. Concentration techniques (sedimentation and floatation) increases the chances of detecting the parasite. Faecal floatation allows for microscopic quantification and identification of helminth eggs in a faecal sample, expressing it as a faecal egg count (FEC) quantified in eggs per gram of faeces (EPG) (MAFF, 1986). A variety of floatation techniques including FLOTAC, Cornell-Wisconsin and the McMaster technique are available. The latter is the most frequently used method in wildlife parasitology (Gillespie, 2006). It is inexpensive and easily replicable. Several faecal floatation solutions are available for separating many helminth eggs. Solutions of saturated

NaCl or sugar can be effective, but $NaNO_3$ is optimal. However, $ZnSO_4$ and $MgSO_4$ are unsuitable for general analyses because they do not isolate many of the nematodes commonly infecting wild primates. Faecal sedimentation method, achieved through gravity or centrifugation allows for the isolation and identification of heavy parasite eggs due to the concentration of the organisms in the sediment, e.g. trematodes that, unlike other helminth, are too heavy to float up in $NaNO_3$ solution (Gillespie, 2006). Different sedimentation techniques by centrifugation including, formalin ether and Merthiolate iodine formaldehyde (MIF) are available and depends on the preservative used on the faecal samples.

2.5.3 Kato- Katz

Kato-Katz thick smear method is a widely used technique in assessing STH including hookworms' prevalence and infection intensities in epidemiological surveys and helminth control programs. Although it is a heap and a simple method, it lacks sensitivity for the detection of low-intensity STHs infections (Knoop *et al*., 2014). It is easy to use in the field, is relatively cheap hence recommended by the WHO for surveillance and epidemiological field survey of STH infections.

Its' sensitivity in determining STH infection from a single stool is limited by day-to-day variation in egg excretion leading to measurement error in estimating the presence of infection. For this reason, recommended examination of several stool specimens collected over consecutive days improves the sensitivity of the test (Booth *et al.*, 2003).

2.5.4. Faecal Cultures

Faecal cultures are widely used for differentiation and identification of morphologically similar eggs of different species. Eggs of different species of gastrointestinal nematodes are similar in size and appearance, making their differentiation extremely difficult. Their third-stage larvae (L3), however, are sufficiently different hence distinguishable between different genera or even species in some cases. The eggs of *A. duodenale* for instance, are morphologically indistinguishable from those of other *Strongylid* nematodes, including *N. americanus* and *O. bifurcum* (Johanna *et al*., 2005). A faecal culture allows the eggs to develop and hatch into infective L3 that have clear morphological characteristics for differentiation. However, reliable identification is time-consuming and requires highly skilled microscopist. Additionally, large-scale application of faecal cultures in epidemiologic surveys requires that stool samples mixed with vermiculite or charcoal kept in closed petri dishes for about one week. At tropical temperature, it leads to the development of maggots and overgrowth of fungi, hence should be performed in duplicate to obtain more reliable results (Johanna *et al.*, 2005).

2.5.5. Serological Method

Invasive methods of nematode diagnosis include post-mortem sampling, endoscopy and serological tests e.g. immunoassays which are based on detection of specific antibodies e.g. IgE. They are easy to use and have high sensitivity compared to direct smear. However, drawing of blood from especially endangered species is little accepted thus hampering the technique. More still, antibody detection cannot distinguish current and past infections (Doenhaff, 2004; Verweij *et al*., 2007).

2.5.6. Molecular Detection

Most nematode parasites are morphologically indistinguishable requiring culturing to hatch eggs into morphologically distinguishable larvae. In addition, microscopic methods do not give species specificity required to discriminate between different species of the same genus. This is especially so for *Strongyles* and *Strongyloides* genus. Given these limitations of microscopic techniques and serological tests, PCR, provides a valuable alternative by specifically detecting nematodes DNA in faecal samples. It offers sensitivity and specificity required to distinguish helminths to species level.

2.6. Helminth Control and Control Challenges

There are various methods applied in control of helminths. They include chemotherapy, improved sanitation and health education. These approaches applied together reduce prevalence and intensity of infections and break transmission cycle.

2.6.1. Chemotherapy

Chemotherapy is the mainstay control strategy for helminth parasites. Single dose or a combination of broad spectrum (since poly-parasitism I common), safe and inexpensive anthelminthic drugs are used in mass drug administration (MDA). They show good results in reducing prevalence and intensity of infections (Onkanga *et al*., 2016). A single dose of Albendazole for instance, gives a 98% parasitological cure for *O. bifurcum* (Ziem *et al*., 2006). A single dose of Albendazole or mebendazole given to pre-school or school-aged children eliminates the STHs; ascariasis and hookworm infections, thereby, reducing STH-induced morbidities, such as anaemia, growth retardation and poor intellectual and cognitive development. However, they have low therapeutic effects for Trichuriasis, thus a second drug, Ivermectin (IVM) or oxantel is used in *Trichuris* endemic areas (Speich *et al*., 2014).

Diethylcarbamazine (DEC), a lymphatic filariasis microfilaricidal drug poses the risk of side-effects (neurological sequalae and fatal encephalopathy) in individuals co-infected with

O. volvulus (Cano *et al*., 2018). Further, DEC can cause severe Mazotti reactions, characterised by intense itching owing to the rapid death of microfilaria in the skin or potential blindness if in the eye (Budge *et al*., 2018). Unfortunately, MDA medicines capable of killing the adult filarial worms are not available. Thus, IVM co-administered with Albendazole that has microfilaricidal properties, provides important collateral benefits simultaneously. Other NTDs such as *Ascaris*, IVM is the only safe drug available for onchocerciasis. It acts on microfilariae that causes the ocular and cutaneous manifestations of the disease and blocks production of new microfilariae by the adult female worms, for at least three to six months post treatment, but does not affect the adult filarial worms directly. Praziquantel (PZQ) used for schistosomiasis is also efficacious against several of the food-borne trematodes e.g. *Fasciola gigantica.*

Overall, MDA has broader impact in improving global health, as a number of drugs used are efficacious against other NTDs. IVM for instance, used for lymphatic filariasis and onchocerciasis control has a potent effect on reducing scabies (Mohammed *et al*., 2012), a mite infestation that in severe cases, result in secondary bacterial infections leading to rheumatic heart disease or glomerulonephritis. Azithromycin used for trachoma, also has beneficial effects against yaws, and the WHO has added the control and elimination of yaws using azithromycin as a target (Hotez *et al*., 2014).

However, continuous administration due to reinfection as transmission persists imposes selection pressure that may lead to drug resistant strains. Drug failure using Albendazole or mebendazole in a single dose has been reported in hookworms (McCarty *et al*., 2014; Webster *et al*., 2014). In addition, the benzimidazole anthelminthic have been shown to result in low cure rates for *Necator americanus* and *Trichuris trichura* (Keiser & Utzinger, 2008; McCarty *et al*., 2014). Filarial helminth, *O. volvulus* adult female worms have shown to be non-responsive to the antifecundity effects of multiple treatments with IVM, resulting in a rapid repopulation of the skin by microfilaria (Osei-Atweneboana *et al*., 2011). MDA monitoring and evaluation (M&E) of drug efficacy is thus imperative. However, standardized M&E are lacking. In addition, MDA targets schools going children, thus the non-targeted population might act as reservoirs. Also, of concern are NHPs as intermediate hosts and reservoirs. Notably, helminth infection is ubiquitous among NHP in densely populated urban centres and occurrence of STHs in NHP the risk of transmission cannot be neglected, as NHPs potentially maintains helminths ensuring continued transmission by re-infection where MDA is being implemented. Heavy reliance on anthelminthic drugs in absence of measure to keep the population free of re-infection

limits effectivity of the approach and increases risk of drug resistance that can render chemotherapy obsolete.

2.6.2. Improved Sanitation and Health Education

Chemotherapy-based control programmes such as MDA remain the main control strategies for helminths. However, in case of STH and schistosomiasis control for instance, it does not kill immature worms and cannot prevent reinfection thus have a temporary effect on transmission (Campbell *et al*., 2014). Infection prevalence and intensity can rapidly return to baseline levels soon after cessation of chemotherapy programmes. This is because helminth eggs /or larvae are able to survive for extended periods in the environment, creating a source for rapid reinfection following chemotherapy (Gray *et al*., 2010). Additionally, small sections of the population usually remain out of reach of chemotherapy programmes; the untargeted population might frequently have a disproportionately heavy burden of infection, thereby serving as a reservoir for reinfection.

Further, NHPs in close proximity with humans and sharing resources such as watering points harbour a variety of helminths and may act as reservoirs. Better sanitation that involves access to clean water, sanitation and hygiene (WASH) practices (Freeman *et al*., 2013), disrupt infection and /or re-infection and transmission of the helminths by reducing contact with soil and /or water contaminated with helminth eggs or larvae. This coupled with behavioral change e.g., use of latrines and footwear ensures chemotherapeutic interventions remain sustainable and bring long-term benefits. The comprehensive strategy for the control and prevention of worm infections jointly published by the WHO and UNICEF, includes the provision of safe water supply and adequate sanitation as a necessary control strategy (WHO, 2004). The WASHED (Water, Sanitation, Hygiene Education, Deworming) framework published by Children Without Worms (CWW) is a sector-based comprehensive approach to STH control that advocates for WASH interventions to break the cycle of STH reinfection (CWW, 2012). However, better sanitation provision, which is a responsibility of national government, is a challenge, particularly in poor nations where diseases are endemic. In addition, use of footwear in disruption of e.g. hookworm that has an alternative route of contraction such as ingestion of larvae in the case of *Ancylostoma duodenale* limits hookworm transmission disruption by footwear. Additionally, high prevalence of STH in NHPs potentially acts as reservoirs of infection. Consequently, the benefits of MDA are jeopardised, a state that needs to be addressed.

2.7. STH Global Elimination and Eradication Strategy

Infectious agents cause a significant proportion of the world's disease burden that lead to mortality or severe morbidity among millions of individuals. NTDs are part of this disease burden. Seven of these NTDs, including bacterial infection *Chlamydia trachomitis*, the causative agent of blinding trachoma, onchocerciasis, LF, schistosomiasis and the STHs, in particular *Ascaris lumbricoides*, *Trichuris trichiura* and hookworm caused predominantly by *Necator americanus*, but also *Ancylostoma duodenale* are amenable to control using safe and inexpensive oral medicine in MDA (Bhutta *et al*., 2014). MDA is based on the principle of preventive chemotherapy (PC), a term introduced by the WHO to define the strategic approach of treating populations infected, or at risk of infection, with these NTDs without individual diagnosis (WHO, 2006).

MDA treatment programmes are designed explicitly to reduce or prevent morbidities in the case of STHs and schistosomiasis and /or interrupt transmission in the case of LF, trachoma and onchocerciasis using anthelminthic drug either alone or in combination (combined therapy). More than 700 million people now receive these essential NTDs medicines annually (Budge *et al*., 2018). MDA is the recommended strategy of the WHO for control or elimination of several NTDs. It has, for instance, called for elimination of LF and trachoma by 2020, which was recently endorsed in the London Declaration on NTDs (Keenan *et al.,* 2013). In addition, it has set a goal to eradicate the burden of parasitic worms in children by 2020. Such extensive coverage will require additional strategies for evaluation focusing on impact and drug efficacy, as well as new diagnostic tools and role of reservoirs in continued transmission. High –coverage MDA in endemic areas aims to prevent and alleviate symptoms and morbidity on the one hand and can reduce transmission on the other, together improving global health. However, maintenance of these pathogens in reservoirs remains a challenge.

2.8. Role of NHPs in Helminth Transmission

Most disease-causing organisms persist in multiple hosts; thus, identification of reservoirs is essential for devising effective interventions. Zoonosis from wildlife present the most significant, growing threat to global health of all EIDs. Of particular concern are NHPs as reservoir hosts. Common human infecting helminths including STHs are also found in free-ranging NHPs near human settlement (Murray *et al*., 2000; Weyher *et al*., 2006). In a study on zoonotic gastrointestinal parasites in NHPs in Kenya, zoonotic helminth species including, *Trichuris* sp., *Strongyloides* sp., *Strongyles* sp., and *Schistosoma* sp., (in order of frequency) were found in old world monkeys (Muriuki *et al*., 1998). *Streptopharagus* sp., and *Strongyloides*

sp., was observed in De Brazza's monkeys (*Cercopithecus neglectus*) in Kenya (Karere & Munene, 2002). *Schistosomiasis mansoni*, a human parasite has been reported in baboons (Muchemi,1992). *Strongyloides stercoralis*, a helminthic parasite responsible for heavy parasitic burden in humans as well as wildlife has a vital definitive host, monkeys, raising serious zoonotic concerns. Natural infection with *S. mansoni* infections in NHPs were reported in different studies in two Rift Valley areas in Ethiopia (Erko *et al*., 2001; Legesse & Erko, 2004). A cestode, *Bertiella* sp., and a trematode, *Dicrocoeliidae* sp., were reported in chimpanzee in Gombe national park in Tanzania (Gillespie *et al*., 2010). Nematodes of the genus *Oesophagostomum* are intestinal parasites, which frequently infect primates (Poldermnan *et al.*, 2010). In Ghana, geographic separation between humans and NHPs infected with *Oesophagostomum* sp., despite apparently conducive environments for zoonotic transmission, suggested that zoonotic transmission is uncommon (Lieshout *et al*., 2005). In Uganda, however, the situation is different and cross-infection is probably more frequent (Ghai *et al*., 2014). Three *Trichuris* species, *T. trichiura, T. sius* and *T. vulpis* are considered zoonotic parasites and a threat to human health (Taylor *et al*., 2001). In fact, *T. trichiura* is easily transmitted between humans and NHPs including monkeys and lemurs (Stephenson *et al*., 2001). Presence of helminths in NHPs poses a threat of helminthiases re-emergence due to reinfection in humans after treatment. With interest increasing in EIDs, the status of zoonotic helminth infection is in need of re-appraisal, considering their impact, in both health and economy. Thus, surveys to assess helminth prevalence among NHPs can play an important role in control strategies because re-emergence of STHs from reservoirs can undo the best eradication efforts. In addition, abundance of NHPs and their sharing of resources with humans e.g. watering points increase the risk of unrecognised transmission of common as well as new or emerging human infective helminths. There is therefore a critical need for health monitoring and identification of new, potentially zoonotic and previously controlled pathogens in wildlife populations, as a forecast measure for IDs and for purposes of development of appropriate control measures. Therefore, this present study sought to investigate helminth infection in common free-ranging NHPs within Kenya urban centres, to better understand the zoonotic risk associated with increased ecological proximity between them and humans and their potential role as reservoirs' facilitating continued transmission where MDA is being implemented.

CHAPTER THREE
MATERIALS AND METHODS

3.1. Ethical Review and Permit

Animal trapping, anesthetisation and sampling was undertaken with approval of and according to the guidelines of the Institute of Primate Research (IPR) Institutional Scientific Review Committee approval number ISERC/04/18. A copy is shown in the Appendix. Permit for trapping the NHPs and collection of samples from them was obtained from Committee of the Department of Veterinary and Capture Services of the Kenya Wildlife Service (KWS), Nairobi.

3.2. Animals Sampling Sites

NHPs faecal samples used in this study were obtained from free-ranging NHPs from four regions: Kakamega, Kisumu and Murang'a Mombasa that are in western, central and coastal regions in Kenya (Figure 7). Previous studies have reported these areas to have high prevalence of STHs in humans (Pullan *et al.*, 2011). However, there is no data on STHs infection in NHPs that are frequently interacting with human in these regions despite their eminent potential as reservoirs for helminths. NHPs were captured at six sites: (a) Mombasa (4° 03′ S, 39°40′E), (b) Kisumu (0° 00′ N, 34°48′E), (c) Murang'a (0° 43′ S, 37° 09′E) and (d) from three peri-urban townships namely Buyangu (0° 19′N 34°57′E), Isecheno (0° 17′N 34°51′E) and Malava (0° 26′ N 43°51′E).

3.3. Animal Trapping and Sample Collection

Animal sampling was opportunistic. Free-ranging NHPs caught were targeted for translocation to wildlife reserves because they were a menace to the public in urban centres or were regarded as pests by small-scale farmers within peri-urban areas. Trapping of animals was done with rectangular traps measuring 1.5 m high and 0.9 m wide, made of iron bars, mesh wire and weighted trap doors designed by IPR staff between June 2018 and September 2018. Briefly, to bait the animals, fresh maize cobs and ripe bananas were used. After their habituation, the traps were set in the evening after the animals had retired to their sleeping sites with the aim of capturing them the next day. Captured NHPs were brought to the central holding site and anaesthetised by administration of a 3:7 mixture of xylazine (2%): ketamine hydrochloride (10%), dosage of 0.1ml/Kg body weight to allow collection of faecal samples and data on gender and age. Qualified veterinarians collected the faecal samples from the rectum. One aliquot of each sample was sampled was stored in 70% ethanol and frozen at -20ºC for molecular examination

and another aliquot in 10% formalin for parasitological examination. Morphology data included colour and length of fur, weight, appearance of the genitals and head shape. Behaviour relationship with the mother, and play were used to assign age. Species and gender of each animal were also recorded.

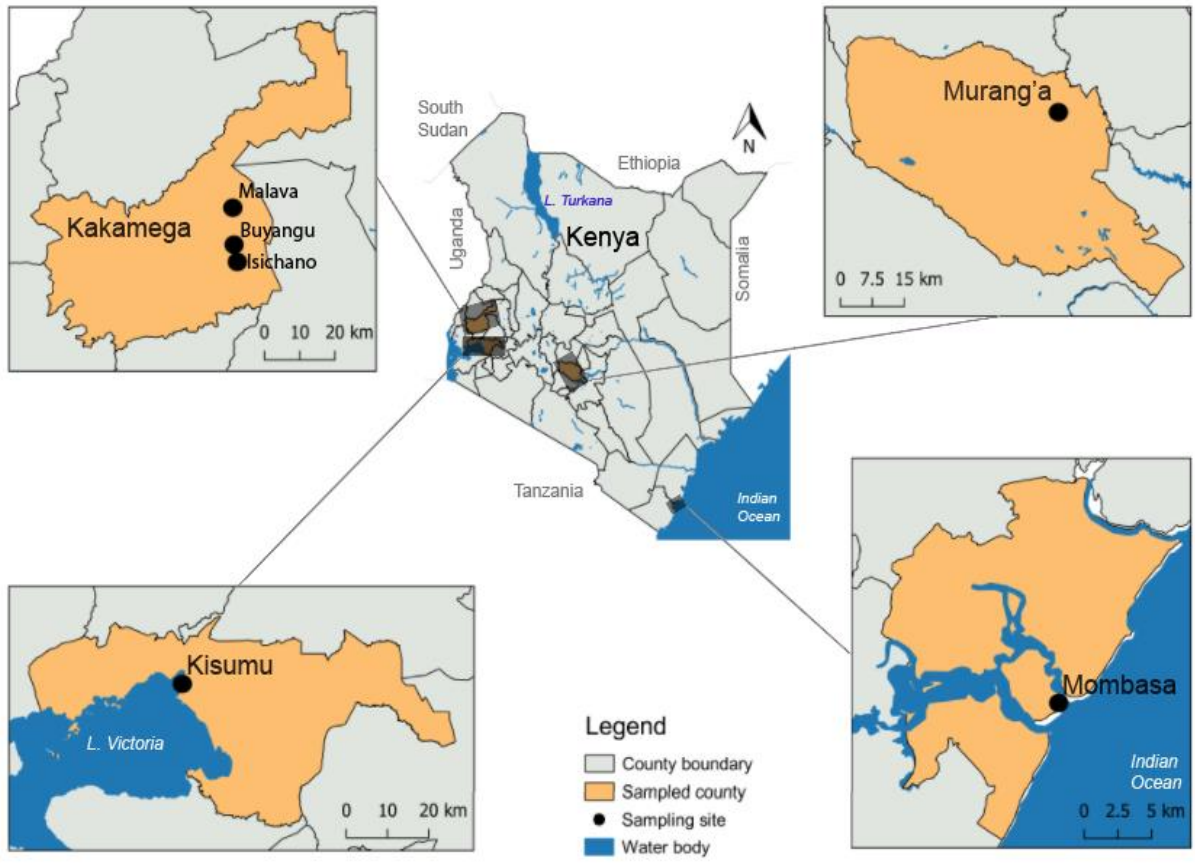

Figure 7: Map showing sampling locations. At the centre is the map of Kenya with the four sampling locations, namely Kisumu, Kakamega, Murang'a and Mombasa shown in brown and blown up from the map. The black solid circles are the sampling sites within the urban and peri-urban centres within the four counties whose boundaries are shown in grey. Other features including water bodies are shown in the legend.

3.4. Parasitological Examination of Faecal Sample

Two parasitological methods for concentration of helminths were used to screen for nematodes: sedimentation and floatation techniques. Although they achieve the same goal, one may be more useful than the other depending upon the species of parasite present and the developmental stage (Garcia *et al.*, 2017).

3.4.1. Formal-Ether Sedimentation Technique

An aliquot of formalin fixed faecal sample was analysed using formal-ether sedimentation technique (Lee *et al*., 2010). Briefly, a gauze mesh filtered 4g of sample into polypot with 10 ml of distilled water for formal ether sedimentation. Subsequently, the filtered solution was centrifuged for 10 min at 500 g using Jouan C422 swing out centrifuge, supernatant discarded and the sediment suspended in 7 ml of 10% formalin. 3 ml of diethyl ether was added to the suspension and mixed thoroughly by shaking. A centrifugation step for 10 min at 500 g followed. Debris and fat, which formed a floating plug, was dislodged using an applicator stick and the supernatant discarded. Subsequently, one drop of the concentrate was transferred to a microscope slide and a drop of Lugol's iodine was added before covering with a 22 mm X 22 mm coverslip. Slides were examined in duplicates under a microscope (Leica DM2000 LED) equipped with a digital camera control unit (Leica DFC 450). Helminths were identified based on egg colour, shape and internal content and representative images captured (Martin *et al*., 2017).

3.4.2. Sheathers Sugar Floatation

For sheathers' sugar floatation, the filtered solution from 4 g of faecal samples was poured into a 15 ml conical tube and the sample volume topped to 14 ml. The sample was centrifuged at x500 g for 10 min and the supernatant poured off. The tube was then filled halfway with sugar solution and mixed by vortexing. Sugar solution was added to have a slight meniscus bulging over the lip of the tube. A cover slip was placed on top of each tube and centrifuged for 10 minutes at x500 g before placing it on a microscope slide. The slides were examined in duplicates under a microscope (Leica DM2000 LED) equipped with a digital camera control unit (Leica DFC 450). Helminths were identified on the basis of egg colour, shape and internal content and representative images captured (Martin *et al*., 2017).

3.5. DNA Extraction

Ethanol preserved faecal samples were snap-frozen in liquid nitrogen and then ground with a pestle to ensure complete breakage of helminth eggs and larvae. DNA was extracted using the Iqama DNA stool Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions with minor modifications. Briefly, 1.4 ml buffer ASL was added to 220 mg of stool sample and vortexed continuously until the sample was thoroughly homogenised. The mixture was then heated at 70º C for 5 minutes before vortexing for 15 seconds and a one-minute centrifugation at full speed (x16000 g) to pellet the stool sample. Subsequently, one inhibitEX tablet was added into 1.2 ml of the supernatant, mixed by vortexing for one minute,

incubated at room temperature for 5 minutes before centrifugation for 3 minutes at full speed to pellet the inhibitors bound to the inhibitEX tablet. 200µl of supernatant was transferred into 1.5 ml micro centrifuge tube containing 15µl of proteinase K, 200µl of buffer AL added, mixed and incubated for 10 minutes at 70º C. Thereafter, 100µl of chilled 100% ethanol was added to the lysate, mixed and transferred to spin columns in collection tubes. This was centrifuged at full speed for one minute, columns transferred to new collection tubes and the filtrate discarded. To wash, 500µl of bufferAW1 was added to the columns and centrifuged for one minute at x16000 g. Washing step was repeated using buffer AW2, and a final centrifugation for three minutes at 16000xg. To eliminate traces of AW2 buffer, the columns were centrifuged at full speed for one minute. Finally, the spin column was placed in a new 1.5 ml micro centrifuge tube and DNA eluted by adding 200µl of elution buffer, incubating for one minute at room temperature, and centrifugation for one minute at full speed. DNA was stored at -20º C.

3.6. PCR Identification of nodular worms

Molecular identification of nodular worm (*Oesophagostomum* spp) involved PCR amplification of ITS2, gene using NC1 forward (5'-ACG TCT GGT TCA GGG TTG TT-3') and NC2 reverse (5'-TTA GTT TCT TTT CCT CCG CT -3') primer pairs (Ghai *et al.*, 2014). PCR was performed in a RotorGene Q thermocycler (Qiagen, Hilden, Germany) using 10µM concentrations of each primer, 4µl of 5X HOT FIREPOL Eva Green HRM Mix (Solis Biodyne, Tartu, Estonia) and 2µl of the DNA template in a 25µl reaction mix. Thermal cycling conditions were as follows: initial denaturation at 95 ºC for 15 minutes, followed by 35 cycles of denaturation at 95 ºC for 1 min, annealing at 55 ºC for 45 secs, and extension at 72 ºC for 45 secs and a final extension at 72 ºC for 5 min. The PCR products were immediately utilised for high resolution melting (HRM) analysis as described (Villinger *et al*., 2017). Briefly, the amplicons were denatured at 95 °C for 1 min, annealed at 40 °C for 1 min and equilibrated at 65 °C for 90 sec, and then increasing the temperature in 0.1 °C increments up to 90 °C with fluorescence acquisition after 2 seconds incremental holding periods. The melting curve profile was then analysed using Rotor-Gene Q series software version 2.1 with fluorescence (melting curve) normalised by selecting the linear region before and after melting transition. Melting temperature (Tm) was interpolated from the normalised data as the temperature at 50% fluorescence. Distinct HRM profiles, normalised in the range of 80-90 °C, were visually determined for each reaction after completion of HRM data acquisition. PCR-HRM products were

further visualised by 2% agarose gel electrophoresis stained with ethidium bromide. Gel readings were compared with corresponding PCR-HRM melting peaks for consistency with HRM analysis.

3.7. Purification of PCR Products

Representative positive amplicons were purified using the QIAquick Gel Extraction Kit (QIAgen, cat.no. 28104, Hilden, Germany), according to manufacturer's instructions. Briefly, the gel was viewed under UV –light and amplified DNA fragments individually excised from the agarose gel with a clean, sharp scalpel and placed in a 2 ml micro centrifuge tube. This was weighed and excess gel cut out so as not to exceed 400 mg per fragment. 3 volumes of buffer QG was added and dissolved by incubation in a water bath at 50 ºC for 10 minutes with intermittent vortexing to ensure the gel slice dissolve completely. One volume of isopropanol was then added to one volume of the gel. Subsequently, the mixture was applied to QIAquick column and centrifuged for one minute. The flow-through was discarded. To wash, 0.75 ml of buffer PE was added to the QIAquick column, centrifuged for one minute and the flow through was discarded. The QIAquick column was centrifuged for one minute at 16,000xg. The QIAquick column was placed in a clean micro centrifuge tube and 50µl of buffer EB added to the centre of the QIAquick column and incubated for one minute followed by a one-minute centrifugation step to elute the DNA. The resulting elute was sent for sequencing outsourced at Inqaba (South Africa).

3.8. Phylogenetic Analysis

Consensus sequences for ITS2 rDNA gene were generated from forward and reverse sequence data using Seqtrace version 0.9.0 (Stucky, 2012) and their identity ascertained by BLAST (Altschul *et al.,* 1990) searches of GenBank (Benson *et al.,* 2005). For species identification, a homology cut-off of 97-100% identity with a GenBank E-value threshold of 1e-130 was used. Sequences generated from this study and ITS2 ribosomal DNA sequences retrieved from GenBank were used for multiple sequence alignments in MAFFT (Edgar, 2004). Evolutionary analyses were performed to determine the relatedness and diversity of the *Oesophagostomum* spp., identified in this study to other nodular worm species described in East Africa. The evolutionary history was inferred using the maximum likelihood method in MEGA7 (Kumar *et al.,* 2016) with bootstrapping at 1000 replicates. Phylogenetic trees were rendered in iTOL (Letunic & Bork, 2019).

CHAPTER FOUR
RESULTS

4.1. Species of NHPs Sampled

A total of 86 NHPs were trapped and sampled. Faecal samples were obtained by rectal swab of individual animal under anesthesia. Of the sampled NHPs, 41 were African green monkeys (AGMs), 30 olive baboons, 10 red-tailed monkeys and 5 blue monkeys (Figure 8 and Table 1). Out of the 86 NHPs sampled 47 were males and 39 females. The largest number of NHPs was sampled in Mombasa (n=30) while the least was sampled in Murang'a (n=7). The animals trapped in Kisumu and Kakamega were 21 and 28 respectively. Adults recorded the highest number sampled (n=49), followed by sub adults (n=29) and juveniles (n=8). Here, juveniles are defined as individuals whose sex organs are not clearly visible, have shorter coat compared to adults particularly on the head and are playful with other juveniles. Sub adults are defined as individuals with a juvenile appearance, but with secondary sex characteristics such as rounding scrotum due to testes enlargement for males and button like pink nipples in females while adults are individuals with fully developed secondary sex characteristics. Olive baboons and red tailed monkeys were only trapped in Kisumu and Kakamega while blue monkeys were trapped only in Kakamega. AGMs were sampled from all regions except Kakamega and majority (29/41) were trapped in Mombasa.

4.2. Parasitological identification of STH Infecting NHPs

Microscopic examination of NHPs faeces after formal-ether sedimentation and sheathers' sugar floatation revealed nematode eggs and larvae in 74/86 samples (Figure 9 A and Table 1). Using these stages, the nematodes were categorised into four genera, namely *Strongyloides* sp., *Ascarid* sp., *Trichuris* sp. and *Enterobius* sp. (Figure 9A). *Strongyloides* sp. eggs are oval, thin-shelled with developing larvae folded inside the eggs, and developed larvae have a short buccal cavity, no sheath and a notched tail (Figure 9A). These were observed in 40 of the 86 animal samples. *Trichuris* sp. were found in 54 of the 86 animals. The eggs are yellow to reddish brown in colour, smooth shelled with bipolar plug. *Enterobius* sp. was detected in only five animals. They have a characteristic asymmetrical shape due to a flattening on one side and thick shell. *Ascarid* sp. eggs are broad, thick shelled, mammilated and brown yellow in colour. They were present in only five of the sampled animals. All the samples were *Oesophagostomum* negative by microscopy.

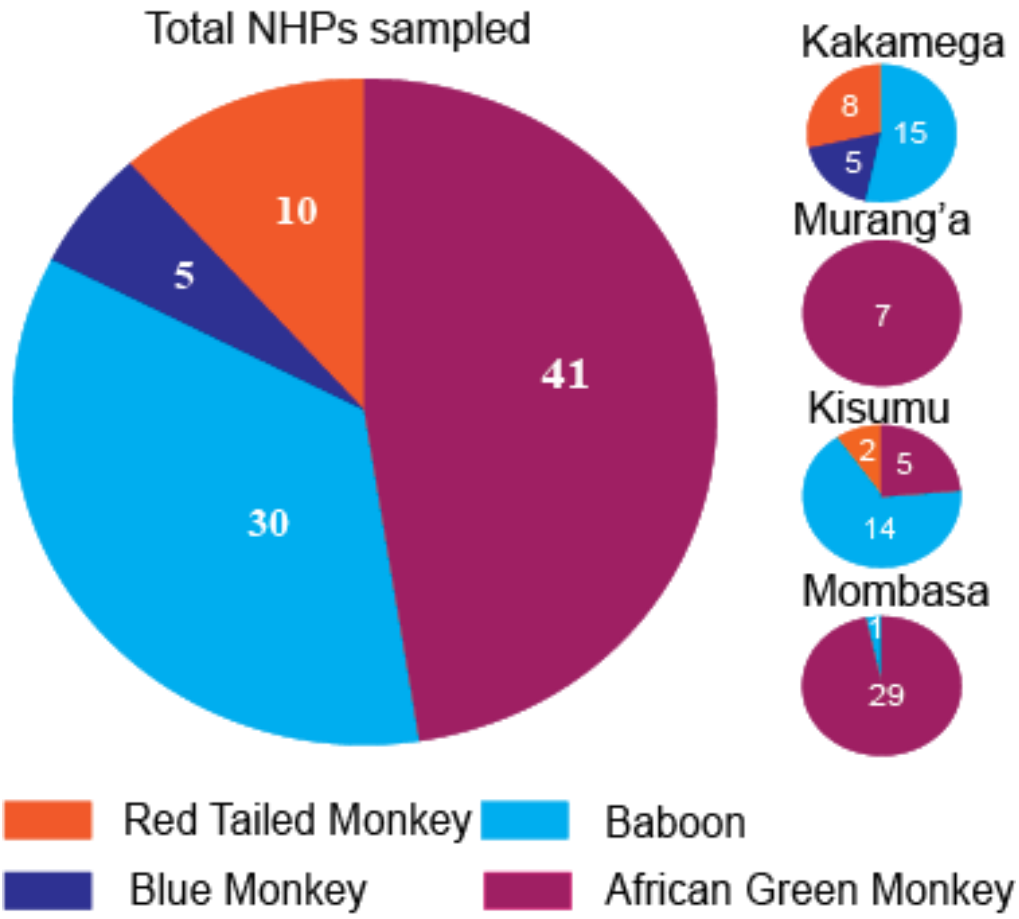

Figure 8: Non-human primate (NHP) species sampled from various urban localities in Kenya. The total number of NHP species sampled, distribution of each species according to sampling urban regions namely Kakamega, Murang'a, Kisumu and Mombasa. The NHP species were African green monkey (AGM), red-tailed monkey, blue monkey and olive baboon as shown by the colour codes, with the number of each animal per sampling region inset in the respective pie charts. Each colour code shows a specific NHP.

Table 1: The various age groups and gender of trapped and sampled animals

NHPs	**Age [Male/Female]**			
	Adult	Sub-adult	Juvenile	Total
African green monkey	24[13/11]	13[11/2]	4[2/2]	41
Olive baboon	17 [5/12]	10[6/4]	3[3/0]	30
Blue monkey	4[1/3]	1[1/0]	0[0/0]	5
Red tailed monkey	4[1/3]	5[3/2]	1[1/0]	10

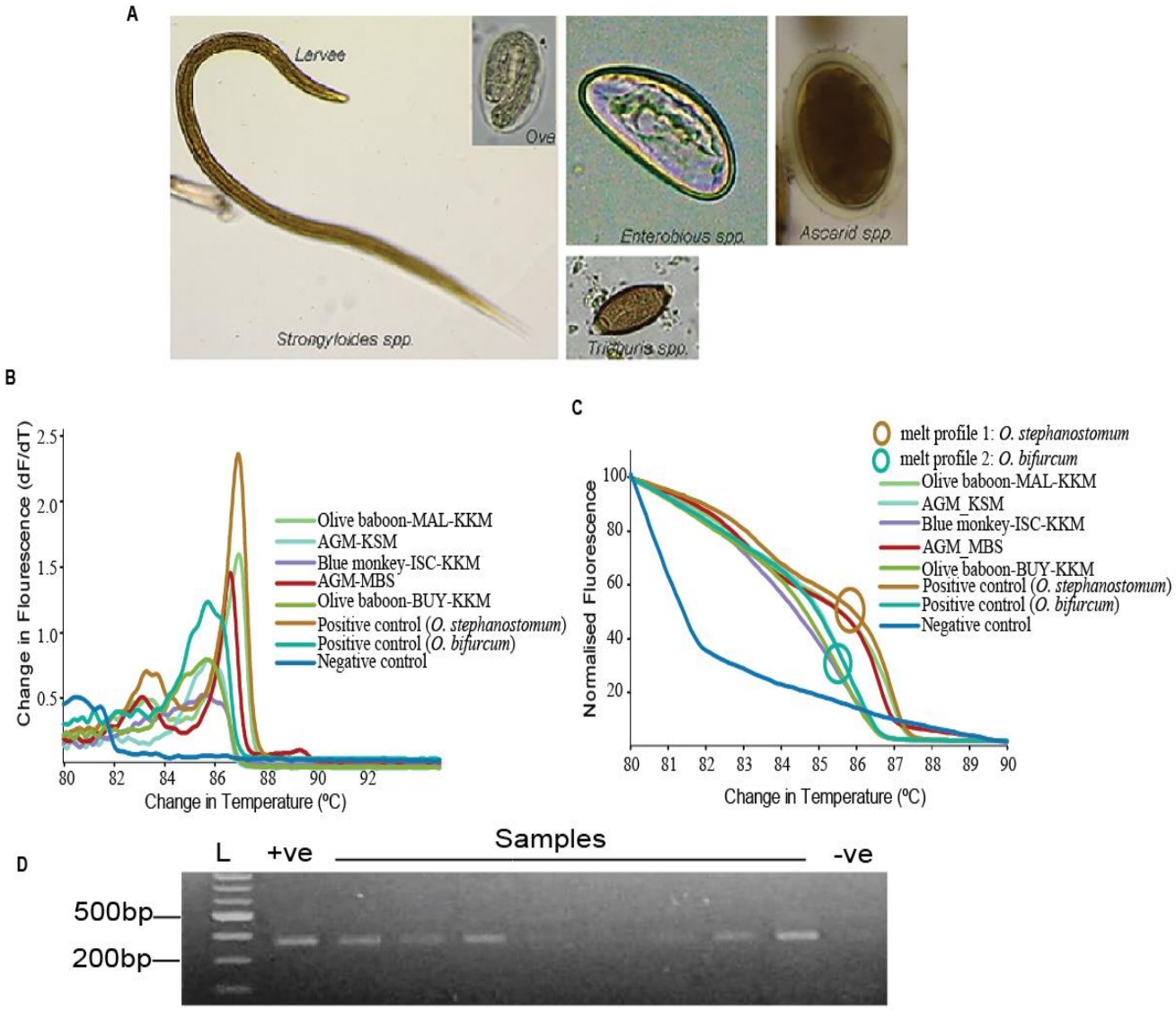

Figure 9: Microscopic and molecular identification of soil transmitted helminths (STHs) in non-human primates (NHPs). **A**. STH eggs and larvae detected in stool samples using microscopy include *Ascarid* spp, *Enterobius* sp., *Trichuris* sp., and *Strongyloides* sp. as shown inset in the various panels. **B.** Representative PCR-HRM melt peaks and **C.** Melt profiles of *O. bifurcum* and *O. stephanostomum* from rectal swabs of sampled NHPs (BUY: Buyangu; MAL: Malava; KKM: Kakamega, and MBS: Mombasa). **D**. Gel electrophoresis size separation of the amplified products(280bp). L, +ve and -ve represents the DNA ladder, positive and negative controls respectively.

4.3. Molecular Identification of nodular worm infecting NHPs

PCR-HRM analysis identified *Oesophagostomum* spp. in 33/86 (38.4%) of the NHPs. Of these, 21 and 23 animals were infected with *O. bifurcum* and *O. stephanostomum* respectively while 11 animals were co-infected with both species ((Figure 9B and C and Table 2). BLAST searches of GenBank upon sequencing confirmed their presence Gel electrophoresis size separation of the amplified products generated amplicons of 280bp. Further confirmation by sequence analysis revealed 97%-99% identity to published sequences.

4.4. STHs Infection in NHPs

AGMs had the most cases of infection, with both *Trichuris* sp., (24/41) and *Strongyloides* sp., (16/41). In olive baboon, *Trichuris* sp., infected 21 out of 30 while *Oesophagostomum* spp infected 25 of 30 animals. *Ascarid* sp., were only found in AGMs (5/41) while *Enterobius* sp., were found in olive baboons (2/30) and red-tailed monkeys (3/10). *Trichuris* sp., *Oesophagostomum* sp., and *Strongyloides* sp., infections occurred in all NHP species as shown in Figure 10 and Table 2.

Table 2. Number of non-human primates (NHPs) infected with soil transmitted helminths (STH) by microscopy and PCR

NHP Species	**Soil Transmitted Helminth (STH)**						
	Trichuris sp	*Enterobius* sp	*Ascarid* sp	*Strongyloides* sp	*O. stephanostomum*	*O. bifurcum*	*Oesophagostomum* Spp
AGM	24	0	5	16	12	9	16
Baboon	21	2	0	16	9	6	11
Blue monkey	2	0	0	3	1	3	3
Red-tailed monkey	7	3	0	5	1	3	3
Total	**54**	**5**	**5**	**40**	**23**	**21**	**33**

4.5. STHs Co-Infections of NHPs

NHPs sampled had co-infections with two, three and four parasites as shown in Figure 10A. Co-infections with *Trichuris* and S*trongyloide*s (n=15) was most common and occurred mostly in AGMs (n=10) from Mombasa. This was followed by *Strongyloides* sp., and *Oesophagostomum* sp., (n=4). They occurred in all the NHPs except AGMs from Kakamega (n=3) and Kisumu (n=1) (Figure 10B). Co-infections with three nematodes involved either one of the *Oesophagostomum* sp., *Trichuris* and *Strongyloides* sp., or *Enterobius* sp., and *Ascarid* sp., (Figure 10C). They occurred mostly in olive baboons (n=8) and AGMs (n=3) while two blue monkeys were co-infected with three nematodes. Three nematodes co-infections did not occur in red-tailed monkey, and were not observed from animals trapped from Kisumu and Murang'a. Co-infections with four nematodes involved *Trichuris sp*., *Strongyloides* sp., *O. bifurcum* and *O. stephanostomum* (n=6) (Figure 11C). They occurred only in olive baboons from all the study regions except Murang'a. Overall, Kakamega had the most cases of co-infections (n=19), followed by Mombasa (n=13) with Kisumu and Murang'a with 9 and 4 NHPs with mixed infections respectively.

4.6. Distribution of Infective STHs According to Sampling Centers

The nematodes infection ranged from 6 to 26 in the four sampling regions. Mombasa had the highest number of infected NHPs (n=26) while Murang'a had the lowest (n= 6), while in Kakamega and Kisumu there were 25 and 17 animals infected respectively (Table 3). Infections with *Trichuris sp., Oesophagostomum spp.* and *Strongyloides spp.* species were highest in Mombasa (n = 23, 13 and 13 respectively) and lowest in Murang'a (n=1). *Ascarid* sp. infections occurred only in Murang'a while *Enterobius* sp., occurred in Kisumu and Kakamega.

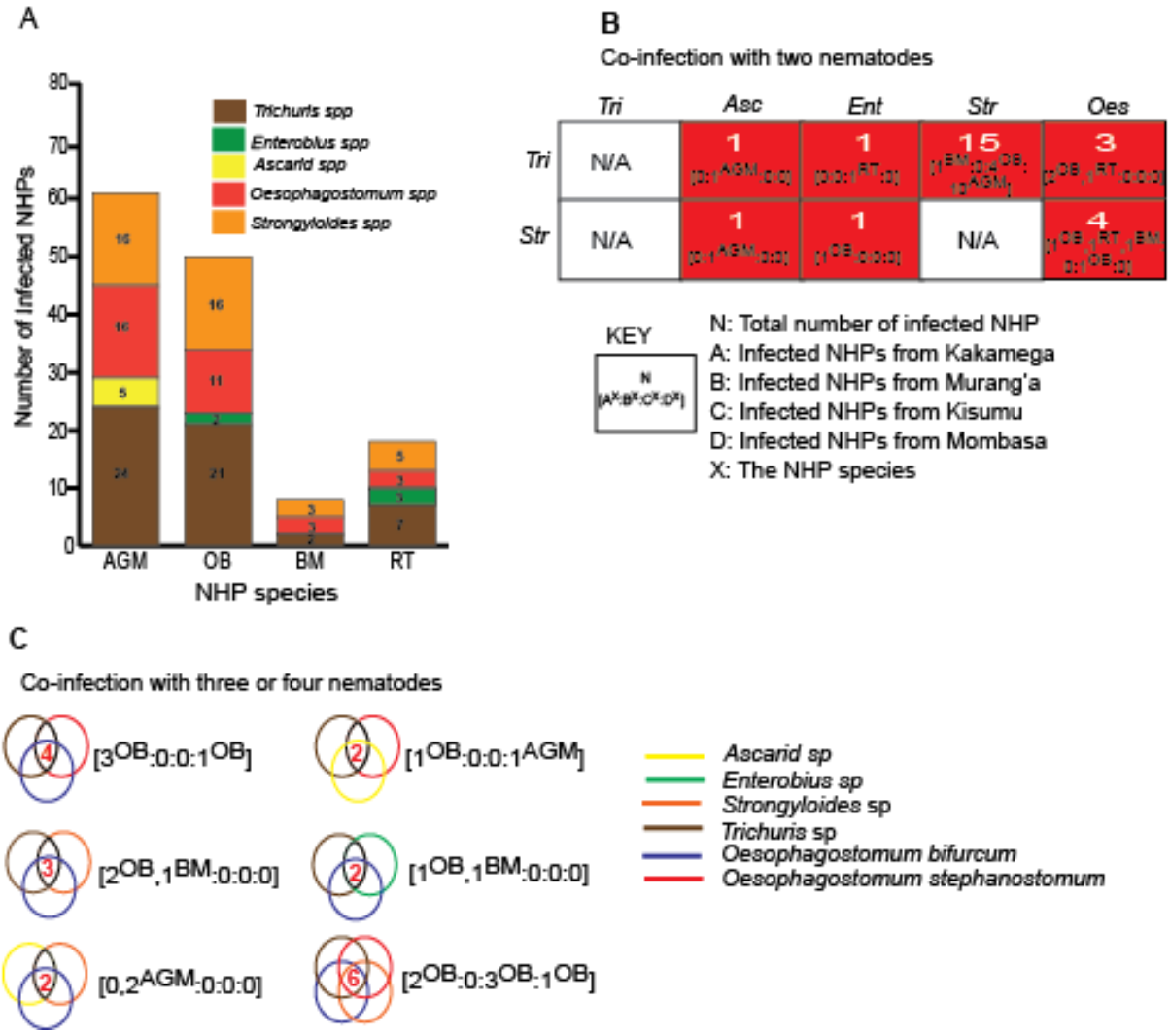

Figure 10: Helminths distribution and co-infections in the sampled non-human primates (NHPs). A. The stacked bar chart shows the number of infections with different species of helminths. AGMs (African Green monkey) had the highest number of infections while blue monkey (BM) had the lowest. In between were olive baboon (OB) and red-tailed (RT) monkeys. **B.** Co-infections with two nematodes shown on vertical and horizontal side of the boxes. The nematodes are *Trichuris* sp. (*Tri*), *Strongyloides* sp., (*Str*), *Ascarid* sp. (*Asc*), *Enterobius* sp (*Ent*) and *Oesophagostomum* sp. (*Oes*). Where there are co-infections are shaded red, with the number of animals co infected shown in white. Further, the site and number of co-infected NHPs were identified are shown according to the key provided. **C.** Co-infections with three or four nematodes as shown by the colour coded circles with the total number of infected NHPs shown at the intersect. The region from which the NHPs were trapped and their number are shown in the key provided in panel B.

4.7. Phylogenetic Analysis

The phylogenetic tree obtained resolved into three distinct clades of *O. stephanostomum, O. bifurcum* and a clade containing the cryptic *Oesophagostomum* spp described in Uganda (Figure 11). The *O. stephanostomum* clade lacked species or geographical sub-structuring; red-tailed monkeys' sequences and a blue monkey sequence formed one sub-cluster while sequences from AGM and red-tailed monkey formed a second sub-cluster. Contrastingly, the *O. bifurcum* clade grouped the baboon and AGM isolates in different sub-clusters. The cryptic *Oesophagostomum* species described from Uganda (Ghai *et al*., 2014) was phylogenetically distinct from both *O. stephanostomum* and *O. bifurcum* identified in this study.

Table 3. Number of non-human primates (NHPs) infected with soil-transmitted helminths (STH) in the four urban centres by microscopy and PCR

Site	**NHPs**	**Soil Transmitted Helminth (STH)**				
		Trichuris spp	*Enterobius spp*	*Ascarid spp*	*Strongyloides spp*	*Oesophagostomum* spp
Kakamega	Baboon	11	2	0	4	5
	Blue monkey	2	0	0	3	3
	Red tailed Monkey	5	1	0	5	2
	Total	**18**	**3**	**0**	**12**	**10**
Kisumu	AGM	1	0	0	0	2
	Baboon	9	0	0	11	6
	Red tailed Monkey	2	2	0	0	1
	Total	**12**	**2**	**0**	**11**	**9**
Mombasa	AGM	22	0	0	12	13
	Baboon	1	0	0	1	0
	Total	**23**	**0**	**0**	**13**	**13**
Murang'a	AGM	1	0	5	4	1
	Grand Total	**54**	**5**	**5**	**40**	**33**

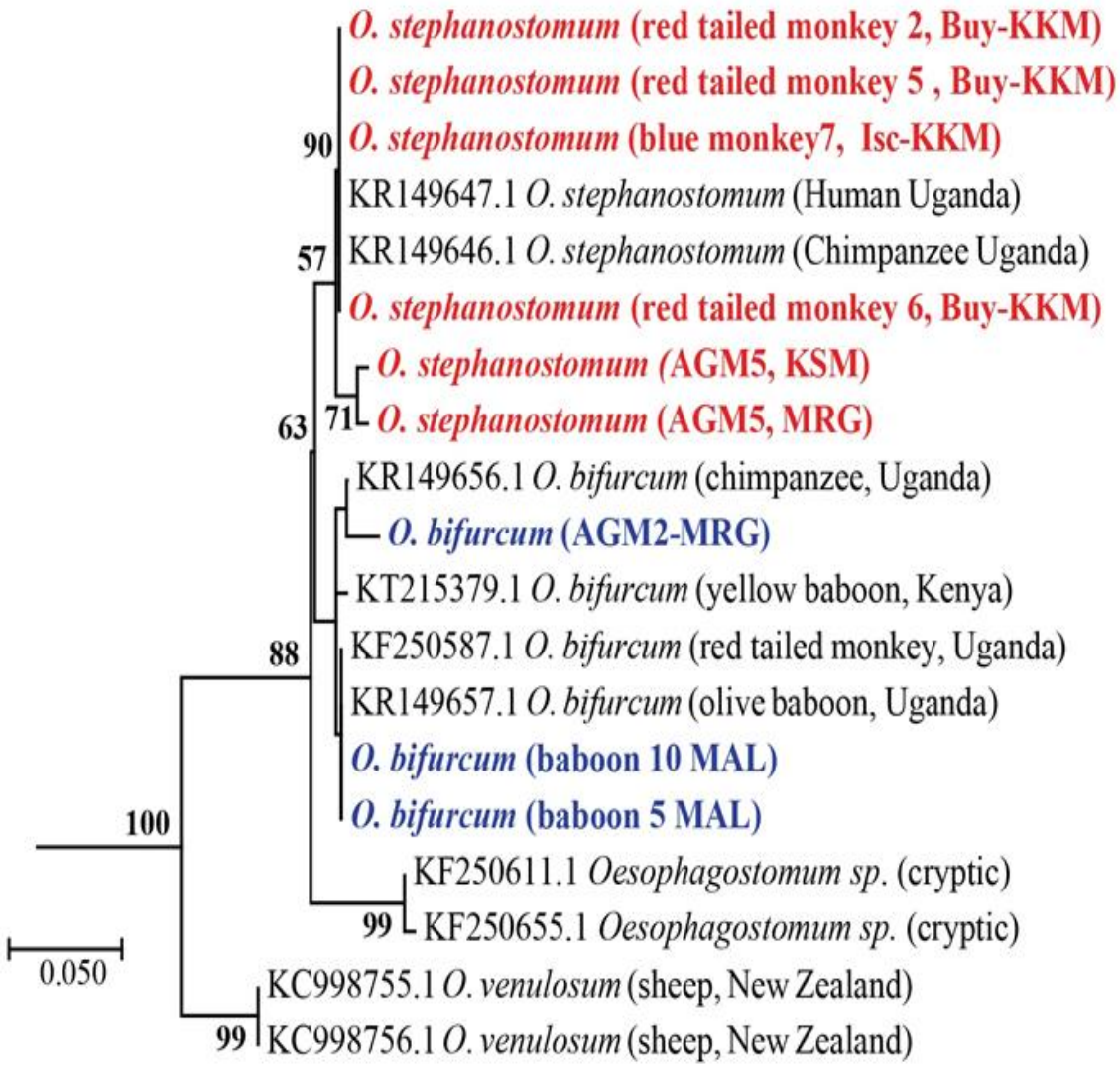

Figure 11: Maximum likelihood tree based on the ITS2 rDNA gene. Sequences generated in this study are shown in bold and correspond to the NHP species, its number and the sampling site (Buy: Buyangu; MAL: Malava; Isc: Isecheno; KKM: Kakamega, KSM: Kisumu; MSA: Mombasa; and MRG: Murang'a). *O. stephanostomum* sequences generated in this study are shown in red while *O. bifurcum* and *T. colubriformis* sequences are shown in blue and green respectively. Bootstrap values are shown as percentages, only values greater than 50 are indicated. Scale bar indicates nucleotide substitutions per site. Evolutionary analyses were conducted in MEGA7.

CHAPTER FIVE
DISCUSSION

5.1. STHs Distribution According to Geographical Region

Parasitological and molecular tests identified diverse STHs infections in NHPs inhabiting the selected urban and peri urban centres in Kenya. The number of STH infections in NHPs was highest in Mombasa (n=26) followed by Kakamega (n=25), Kisumu (n=17) and Murang'a (n=26). By comparison, the prevalence of all STHs in human population in Kenya is highest in coastal and western regions with hookworm, *Ascaris lumbricoides* and *Trichuris trichiura* predominantly attributed to these infections (Brooker & Michael, 2000; Masaku *et al.*, 2017). The current findings are also consistent with (Brooker *et al.*, 2015) that reported high prevalence of STHs in Kwale-Mombasa and Busia-Kisumu. Prevalence in these regions exceeds 20%, the WHO cut off in administration of helminthic drugs in MDA programmes.

NHPs sampled from Mombasa had the highest number of infections with *Trichuris.* One explanation for higher cases of *Trichuris* sp., in this region could be a coastal habitat that is an ideal environment condition including appropriate humidity for egg development. Kakamega had the second highest number of infections with STHs in this study. This is in agreement with another study that assessed prevalence of three STHs (*Ascaris lumbricoides,* Trichuris *trichiura* and hookworm) infections in school children in Kakamega (Ngonjo *et al.,* 2016). The overall STHs infections were 52.1% with *Trichuris* recording the second highest prevalence after hookworm. This was higher than what was reported in a study in Kisumu East (17.4%) (Brooker & Michael, 2000). Similarly, number of STH infections in Kisumu was lower than that observed in Kakamega. However, Mwandawiro *et al*. (2013) reported high prevalence of *Ascaris lumbricoides* and *Trichuris trichura* in Rift valley-Kenya in human population.

Consistent with previous studies (Pullan *et al*., 2011; Kamande *et al*., 2015) reporting pockets of high prevalence of *A. lumbricoides* and *T. trichiura* in central Kenya, the study observed *Ascarid* sp., only in Murang'a. *Ascaris lumbricoides* and *T. trichura* have restricted distributions, with highest prevalence reported in central and western Kenya, where environmental conditions favour their survival and transmission. They have also been shown to be prevalent in south eastern Uganda, north eastern Tanzania and Burundi (Tuyizere *et al*., 2018). Information on *Strongyloides* sp., compared to other major STHs, namely *Ascaris lumbricoides*, hookworms and *Trichuris trichiura*, in human population in Kenya are lacking. They have however been reported in NHPs (Munene *et al*., 1998; Mbora & Munene, 2006; Akinyi

et al., 2019). The prevalence observed in cross-sectional studies performed in sub-Saharan Africa, (Knoop *et al*., 2008; Becker *et al*., 2011; Tuyizere *et al.*, 2018) shows *Strongyloides* sp., to be highly prevalent in human population. *Enterobious vermicularis*, a zoonotic nematode considered a rare parasite was also observed in baboons and blue monkeys. It has also been reported in NHPs (Munene *et al*., 1998; Akinyi *et al*., 2019). However, studies of its prevalence in human population in Kenya are lacking. A possible explanation for the lack of data on *Stronyloides* sp., is the diagnostic methods most commonly used. Direct smear or Kato-Katz, used for major STH detection, have low sensitivity for *Strongyloides sp.,* and may fail to detect it altogether while other more sensitive techniques such as Koga Agar plate culture consume more resources and time and hence, are rarely used in potentially endemic settings of resource poor countries. Presence of *Oesophagostomum* spp*, Enterobius vermicularis* and *Strongyloides* sp., in NHPs in these regions suggest possible circulation in human population inhabiting these centres and should not be ignored in STHs control.

A national school-based deworming programme was started in 2012 in four regions (Western, Nyanza, Rift Valley and Coast) endemic for STHs and schistosomes in Kenya. The programme aimed at reducing infection and associated morbidity. The change in prevalence and intensity of these infections as monitored from 2012 to 2017, showed highest relative reduction in both prevalence and intensity in hookworm infection followed by *A. lumbricoides* (Mwandawiro *et al*., 2013). It indicated that MDA using the annual single-dose oral Albendazole™ was efficacious against hookworm and *A. lumbricoides* infections but not *T. trichiura*. This finding is in agreement with previous studies (Keiser & Utzinger, 2008; Zerdo *et al*., 2016). A study in southern Ethiopia evaluating rate of re-infection with STH among school-aged children after MDA, (Zerdo *et al*., 2016) recorded higher re-infection rates particularly for *A. lumbricoides* compared to other species. In Kenya, Mwandawiro *et al*. (2019) recorded similar results. This finding parallel that of Jia *et al*. (2012) who in their systematic review of STH re-infection after treatment, evaluated 24 studies and observed that re-infections occur rapidly after treatment particularly for *A. lumbricoides* and *T. trichiura*. Whilst the studies demonstrated that MDA was effective in reducing STH prevalence and intensity, the re-infection rate remain problematic and may jeopardise the sustainability of control efforts against STHs. Reasons for the high rate of infection remain unclear, but the possibility of contracting infective pathogens could be rampant, and so could also be in NHPs co-existing in these regions. This is a possible explanation for high infection numbers with *Trichuris* in NHPs in the current study. Moreover, ova of *Trichuris* species have a tough outer coat that enables them to

resist adverse external environmental conditions which enhances their survival and probability of transmission. Additionally, the genetic polymorphisms in β-tubulin, which cause benzimidazole resistance in livestock parasites, have been found in *T. trichiura* and *N. americanus (*Jia *et al*., 2012*)*. Part of the cause of the low efficacy of Albendazole in *T. trichiura* and *N. americanus* may be due to benzimidazole-resistant genotypes in these parasites. Presence of these parasites in NHPs living in close proximity to human poses a risk of infection and re-infection despite MDA. This may in return create selective pressure resulting in development of drug resistance. Therefore, there is need for additional control approaches possibly involving transmission-blocking to sustain the chemotherapeutic gains of MDA and accelerate attainment of elimination of these NTDs as a public health problem in Kenya.

5.2. Distribution of STHs among NHPs

In the current study, AGMs had the most cases of infection with STHs while blue monkey had the least cases of infection. This may be due to behavioral factors; blue monkeys are arboreal while AGMs are terrestrial. It is possible that terrestrial primates contact soil more frequently increasing the chances for contact and contraction of the infective stages of STHs. High prevalence of helminth infections is observed in terrestrial NHPs (Poulin & Morand, 2004; Nunn & Altizer, 2006; Ghai *et al.,* 2014). However, and to the best of our knowledge, there are no studies comparing infection rates among different species of NHPs in Kenya. In addition, majority of AGMs (29/41) were trapped and sampled from Mombasa where overall prevalence in STHs in Kenya is high. Although Olive baboons are also terrestrial, their relatively lower numbers of STH infection compared to AGMs may be a consequence of low sample size, and therefore strong inferences cannot be made. Because most NHPs live in troops, and more often forage in common areas, one would expect infections within a troop to be common if the troops forage in infected regions. Moreover, frequent recurrence of STHs in the same locations may facilitate accumulation of their infective stages and could result in re-infection and high prevalence. And since there are no control strategies in free-ranging NHPs, most infected animals remain infected, increasing risk of transmission among members of their troops.

5.3. Demographic covariates of STHs infection in the NHPs

Data on demographic associations with gastrointestinal parasite infections of NHPs vary widely in their findings (Müller-Graf *et al*., 1997; Mutani *et al*., 2003). In this study, more males than females were infected with no significant difference. Older individuals have been shown to have high prevalence of gastrointestinal parasitic infections (Foerster *et al*., 2015; Friant *et al*., 2016). Consistent with these findings, adults in this study had higher rates of infections than sub adults and juveniles (Table S1). A possible explanation for this is that adults contact the environment much longer and thus helminth infective stages and consume more food, increasing their risk of infection (McCabe *et al*., 2014).

While most studies on NHP parasite species rely on faecal samples collected after defecation, challenges in interpretation of results may arise due to contamination on the ground or collection of several samples from the same animal. In the current study, stool samples examined could be traced back to the individuals having collected the sample from the rectum of the animals, recorded data on the NHPs species, age and gender. Consequently, the determined number of infections are an accurate presentation of infections. In addition, while most studies have used microscopy in NHPs parasites studies, PCR approach allowed detection of additional worm, *Oesophagostomum* sp, which could not be detected by microscopy. Absence of these nematodes via microscopy might be because of low worm burden and hence low egg output rather than being parasite free. Previous studies have shown that variations in parasite infection among individuals, age and sex categories are linked to the condition of the host (Holt *et al*., 2003; Nunn & Altizer, 2006). However, while stress is known to be a factor influencing parasitic infection (Chapman *et al*., 2007; Akinyi *et al*., 2019), host condition is difficult to quantify and compare in natural populations especially free-ranging NHPs.

5.4. Coinfections and Zoonotic potential of STHs

Majority of NHPs examined in this study harboured at least one parasite. Co-infections with two parasites mostly involved the *Trichuris* sp., and the *Strongyloides* sp., while more than three parasite infections involved one of the two *Oesophagostomum* sp., *Trichuris* sp., and *Strongyloides* species. Co-infections in NHPs have also been observed by others (Munene *et al*., 1998; Karere & Munene, 2002). In humans, multiple infections with *Ascaris lumbricoides* and *Trichuris trichiura* has been reported in children in Busia, Kisumu (Brooker & Michael, 2000) while mixed infections with *Ascaris*, hookworm, *Trichuris* and *Taenia* species in children was reported in rift valley-Kenya (Ruto & Mulambalah, 2019). The present results demonstrate that urban and peri-urban NHPs in Kenya are infected with both *O. stephanostomum* and

O. bifurcum. This is in agreement with Cibot *et al.* (2015), who also recorded mixed infections with the two species from chimpanzees in Uganda. The strong association between the *Trichuris* and *Strongyloides* sp., in this study may reflect their abundance in faecal samples. In addition, a helminth induced immunosuppression on infection, caused by infection with one intestinal helminth species may explain the spread of a secondary infection (Cox, 2001). The distinct spatial niches of the worms within a host also explains the nematodes co-infection phenomena. *Ascaris lumbricoides* for instance predominantly reside in the jejunum while *Trichuris trichura* resides in the cecum reducing levels of resources competition between the two worms. However, to date, little is known about co-infection dynamics between helminths in wild primates.

Strongyles are helminthic nematodes commonly reported in primates' parasite studies. Among them, *Oesophagostomum* sp., are often overlooked as a serious zoonotic concern in East Africa, especially Kenya due to their geographical occurrence in West African countries. PCR-HRM and sequencing demonstrate that urban and peri-urban NHPs in Kenya are infected with both *O. stephanostomum* and *O. bifurcum*. Mixed infection with both species observed in this study parallels Krief *et al*. (2010) and Cibot *et al*. (2015). Consistent with Ota *et al.* (2015), phylogenetic analysis revealed no evidence of an *Oesophagostomum* sp., described by Ghai *et al*. (2014) and recently by Cibot *et al.* (2015). This cryptic species is human infective and was also found in five monkey species (Ghai *et al*., 2014). It differed from both *O. stephanostomum* and *O. bifurcum* in ITS2 nucleotide arrangement. Presence of this new taxon in Uganda underscores the importance of local-scale research for potentially hidden genetically diverse nematodes even within well-described genus of parasites that are known to infect humans. Possibly, this new taxon is more adaptive to certain ecological factors and hence its absence in the current study.

Phylogenetic reconstruction of nodular worm isolates demonstrated separation of *O. stephanostomum* sequences into a sub-cluster consisting of isolates from red-tailed monkeys and blue monkeys in Kakamega and a sub-cluster consisting of sequences from AGMs sampled in Kisumu and Murang'a indicating that *O. stephanostomum* is neither geographical nor host species-based. In addition, clustering of *O. stephanostomum* detected in this study with an isolate from human (accession number KR149647.1) indicates close evolutionary relatedness and therefore suggests the potential for this parasite to infect both humans and NHPs. This finding concurs with Cibot *et al*. (2015) reports of human and NHP infection with *O. stephanostomum*, suggesting increased risk of transmission for this helminth between primate species. *O. bifur-*

cum sequences from olive baboons in this study formed a monophyletic cluster with *O. bifircum* sequences from other NHP species retrieved from Genbank. However, the *O. bifurcum* sequence from a chimpanzee sampled in Uganda clustered with sequences recovered from an AGM in Kenya. The two were evolutionarily divergent from the rest of the sequences within this cluster. This points to potentially new host species since *O. bifurcum* has not been previously reported in AGMs in Kenya and is not commonly described in chimpanzees. Because transmission occurs via the ingestion of the infective third-stage larvae present in contaminated food or water, oesophagostomosis is a potential zoonotic risk when infected NHPs and humans share the same habitats. Therefore, intervention strategies to combat oesophagostomosis need to factor NHPs as potential reservoirs.

5.5. Potential Role of NHPs as Reservoirs in STH Control

STHs of NHPs in rural and captivity settings have been well-documented using mostly microscopic methods (Munene *et al*., 1998; Muriuki *et al*., 1998; Murray *et al*., 2000; Gillespie *et al*., 2005; Lim *et al.,* 2008; Kouassi *et al.,* 2015), but in Kenya, little is known about STHs in urban inhabiting NHPs. This gap exists despite their eminent potential as reservoirs of STHs. The WHO has set a goal to eradicate the burden of parasitic worms by 2020 using a combination of broad spectrum and safe anthelminthic drugs in mass deworming targeting pre-school and school going children. For such targeted efforts a comprehensive knowledge of the presence, distribution pattern and transmission dynamics of these helminth infections including their reservoirs needs to be assessed thoroughly.

The present study demonstrates that NHPs within Kenyan urban and peri-urban centres are hosts to different STHs. All helminth parasites identified in this study have zoonotic potential and are soil-mediated. In particular, *Trichuris* spp, the most commonly observed STHs in this study, causes clinical disorders in people worldwide and is one of the predominant human infective STH with high prevalence in coastal and western regions of Kenya. *Trichuris* sp. has been detected in different primate species in Kenya (Munene *et al*., 1998; Karere & Munene, 2002, Mbora & Mcpeck, 2009; Akinyi *et al.*, 2019). Eggs of *Trichuris* sp., have been experimentally transmitted from NHPs to humans (Monteiro *et al.,* 2007) providing evidence of their zoonotic potential. *Trichuris* host diversity was recently investigated and single taxa were found to infect both humans and wild primates (Ghai *et al*., 2014). Therefore, a complex anthropo-zoonotic transmission cycle may be maintained in the study regions. Likewise, cross-infection with ascarids from animals to humans is possible (Strait *et al*., 2012). *Ascarid* sp., was observed only in AGMs. It is interesting to note that it was observed only in AGMs from

Murang'a, central Kenya consistent with a survey by Pullan *et al.* (2011) that reported pockets of high prevalence of *A. lumbricoides* in human population in central Kenya. Infections by *Ascarid* sp has been previously reported (Lilly *et al.,* 2002; Gillespie *et al.,* 2010) in ape populations that share habitats with human. This suggests that there is a high risk of cross transmission of the *Ascarid* sp., and NHPs in this region are potential reservoirs. MDA monitoring and evaluation studies and those evaluating re-infection have shown low efficacy of Albendazole on *Trichuris* sp and its high re-infection rate together with *Ascaris lumbricoides.* Presence of infected NHPs in these regions may play a role in maintaining transmission by enabling re-infection. Continued administration of antihelminths in absence of transmission blocking may thus result in selective pressure and eventually drug resistance jeopardising MDA as a control strategy.

A zoonotic nematode, *E. vermicularis,* identified in this study has also been isolated in captive baboons in Kenya (Munene *et al.,* 1998). However, data on its prevalence in human population is lacking. Being a soil mediated nematode with a simple life cycle that requires no intermediate host, its presence in NHPs living in close proximity to humans poses a risk of continued transmission to human where control deworming is being implemented.

High prevalence of infections with S*trongyloides* sp., has been reported in NHPs (Rothman *et al.,* 2008; Freeman *et al.,* 2013). Consistent with these reports, *Strongyloides* sp., was observed in the present study across all the NHPs sampled. A peculiarity of *Strongyloides* spp. larvae is their ability to penetrate the host's skin (Gholami *et al*., 2015). Because *Strongyloides* sp., have both direct and indirect life cycle, NHPs infected with this parasite increase the risk of transmission to humans in the same habitats and/or living in close proximity. Along with *Strongyloides* spp., infective strongylid larvae develop in moist substrate. Yet, cases of human infection with *Oesophagostomum* have been rarely reported in Kenya. Transmission pathways of zoonotic helminths detected in the present study are primarily related to soil. The high spatiotemporal overlap between NHPs and humans in the studied regions coupled with presence of various STHs present factors that could increase the risk of transmission between the two primate species. In addition, they may act as reservoirs of these STH infections. To mitigate the risks of possible development of drug resistance there is need for additional control approaches possibly involving transmission-blocking to sustain the chemotherapeutic gains of MDA and accelerate attainment of elimination of these NTDs as a public health problem in Kenya.

Transmission potential of the oesophagostomes between NHPs and humans under natural conditions has been under considerable debate. Lieshout *et al*. (2005) reported that *O. bifurcum* from humans and olive baboons are distinct and are unable to cross infect. However, Guillot *et al.* (2011) and Cibot *et al.* (2015) recently reported human cases with *O. stephanostomum* infection, considered a parasite of NHPs in the Sebitoli area of Kibale by DNA amplification. Previous studies (Krief *et al.,* 2010; Kooriyama *et al.,* 2012; Cobit *et al.,* 2015; Ota *et al.,* 2015) reported *Oesophagostomum* sp., in NHPs and humans living in sympatry and suggested an increased risk of transmission for this helminth between primate species. Because transmission occurs through ingestion of the infective third-stage larvae present in contaminated food or water, oesophagostomosis is a potential zoonotic risk when humans and non-human primates share the same habitats. Additionally, due to the severity of the clinical consequences of oesophagostomosis, it should not remain a neglected area of public health.

In sum, the potential role of NHPs as reservoir of STHs and hence play a role in transmission dynamics is likely. Here, control strategies applied, namely sanitation and MDA disrupt the life cycle of STHs, hence breaking continuity and reducing risk of infection. However, NHPs, alternative hosts of these nematodes that are not subjected to any control approaches, act as reservoirs ensuring continuity of the soil transmitted helminths life cycle. Consequently, infective worms are present despite control in human, sustaining continued infection and/or re-infection. Therefore, the role of STHs infected NHPs in continued transmission is potentially real, since there has been empirical evidence of cross infection. Illustration of their possible role has been summarised in Figure 13 below.

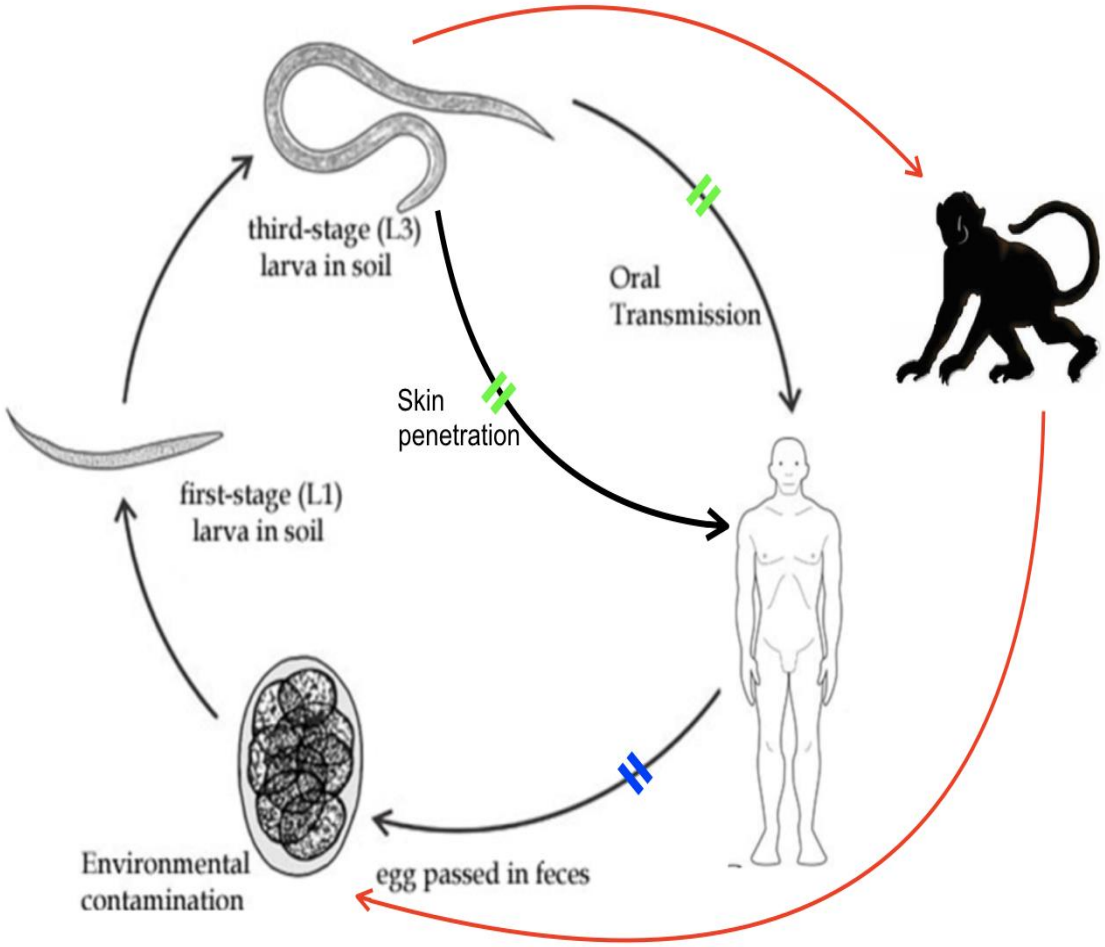

Figure 12: Potential role of non-human primates as reservoirs of STHs. Common human infecting helminthic nematodes are controlled using various approaches, including sanitation (green bars) and drug (blue bars) disrupting the life cycle. However, NHPs offer an alternative route of human infection, ensuring an undisrupted life cycle of the nematodes. The NHPs therefore act as reservoirs that sustain continued survival and risk for infection and re-infection in man. This role in transmission is crucial in control.

CHAPTER SIX
CONCLUSIONS AND RECOMMENDATIONS

6.1. Conclusions

This study demonstrated that urban restricted NHPs in Kenya are infected with diverse nematodes, including *Strongyloides* sp., *Enterobius* sp., *Ascarid* sp., *Trichuris* sp., and nodular worm, *O. stephanostomum* and *O. bifurcum,* and co-infections are common. These helminths are zoonotic and have a high potential for cross transmission. Thus, NHPs can act as reservoirs and potential source of human helminthiases in densely populated urban centres.

It also illustrates the possibility of utilising PCR-HRM analysis to efficiently differentiate between *Oesophagostomum* spp. without the need for sequencing as earlier described for hookworms. PCR-HRM differentiated *O. stephanostomum* and *O. bifurcum* co-infecting NHPs as confirmed by sequencing indicating its utility as a non-subjective approach to supplement sequencing for accurate characterisation of nodular worms.

Clustering of *O. stephanostomum* in this study with an isolate from human indicates close evolutionary relatedness and therefore suggests the potential for this parasite to infect both humans and NHPs. Two evolutionarily divergent *O. bifurcum* sequences from chimpanzee sampled in Uganda and an AGM from Kenya points to potentially new host species since *O. bifurcum* has not been previously reported in AGMs in Kenya and is not commonly described in chimpanzees. Because transmission occurs via the ingestion of the infective third-stage larvae present in contaminated food or water, oesophagostomosis is a potential zoonotic risk when infected NHPs and humans share the same habitats. Therefore, intervention strategies to combat oesophagostomosis need to factor NHPs as potential reservoirs.

The infection pattern between male and females and among age groups is not surprising considering the NHPs living behavior; feeding, energy and nutritional stress in males to maintain social dominance in addition to living in either the same or sympatric troupes. Infection in any age group or sex thus pose infection risk to humans.

This knowledge is important in appropriate deployment of current control strategies. More importantly, the elimination and eradication strategies will have to factor the potential role of infected NHPs in transmission dynamics, and could potentially necessitate the development of novel alternative and/or re-tooling current control strategies. In addition, parasitic infection with *Oesophagostomum* species is considered endemic in West African countries Togo and Ghana (Lieshout *et al*., 2005; Arizono *et al*., 2012), however it may be present across Sub-

Saharan Africa including Kenya. Thus, implementation of effective control measures for STH infections should seriously consider this zoonotic implication.

Characterising the parasites of wildlife hosts sharing the same habitat with human provides useful insights into the mechanisms underlying variation in parasitism as well as potential reservoirs of zoonotic parasite. Elimination and eradication of STH infections using MDA by 2020, as targeted by WHO requires information about the efficacy of the MDA in light of potential reservoirs. NHPs are increasingly found in urban environments bringing closer contact with human and increasing chances of infections. Presence of STHs in NHPs in frequent contact with humans in densely populated Kenyan urban centres as demonstrated in this study potentially jeopardises the effectiveness of MDA by maintaining continued transmission in human. Specifically, NHPs can act as reservoirs, with their interactions with human potentially enabling re- or infections, jeopardizing current control approaches. Consequently, the use of anthelminthic in MDA, the major control strategy might result in development of drug resistant strains rendering it obsolete.

6.2. Recommendations

All helminths detected in this study are zoonotic and have high potential for cross transmission. The WHO has set a goal to eradicate the burden of parasitic worms by 2020 using a combination of broad spectrum and safe anthelminthic drugs in mass deworming, mass drug administration (MDA). However, MDA alone in the absence of breaking the transmission cycle is not enough. Presence of zoonotic helminths in NHPs living in close proximity to humans may act as reservoirs. We thus recommend that control measures being implemented, consider this aspect of transmission. Further, PCR approach allowed the identification of two species of *Oesophagostomum* infecting the NHPs in the study areas. This finding raises the need for better public health awareness of oesophagostomiasis in the study areas. Previous studies have reported *Oesophagostomum* sp. infection in primates from Uganda and their potential zoonotic importance (Krief *et al*., 2010; Ghai *et al*., 2014; Cibot *et al*., 2015; Ota *et al*., 2015). The *O. stephanostomum* known to infect primarily the NHPs was shown to infect humans in Uganda (Cibot *et al*., 2015). In addition, a previously un-identified species was reported in the same country (Ghai *et al*., 2014). Thus, *Oesophagostomum* sp could be common in East Africa and its prevalence in NHPs, other wild animals and humans should be investigated further. Further studies are required for phylogeny analysis of *Oesophagostomum* sp from non-human primates and human to ascertain zoonotic importance in Kenya.

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APPENDICES

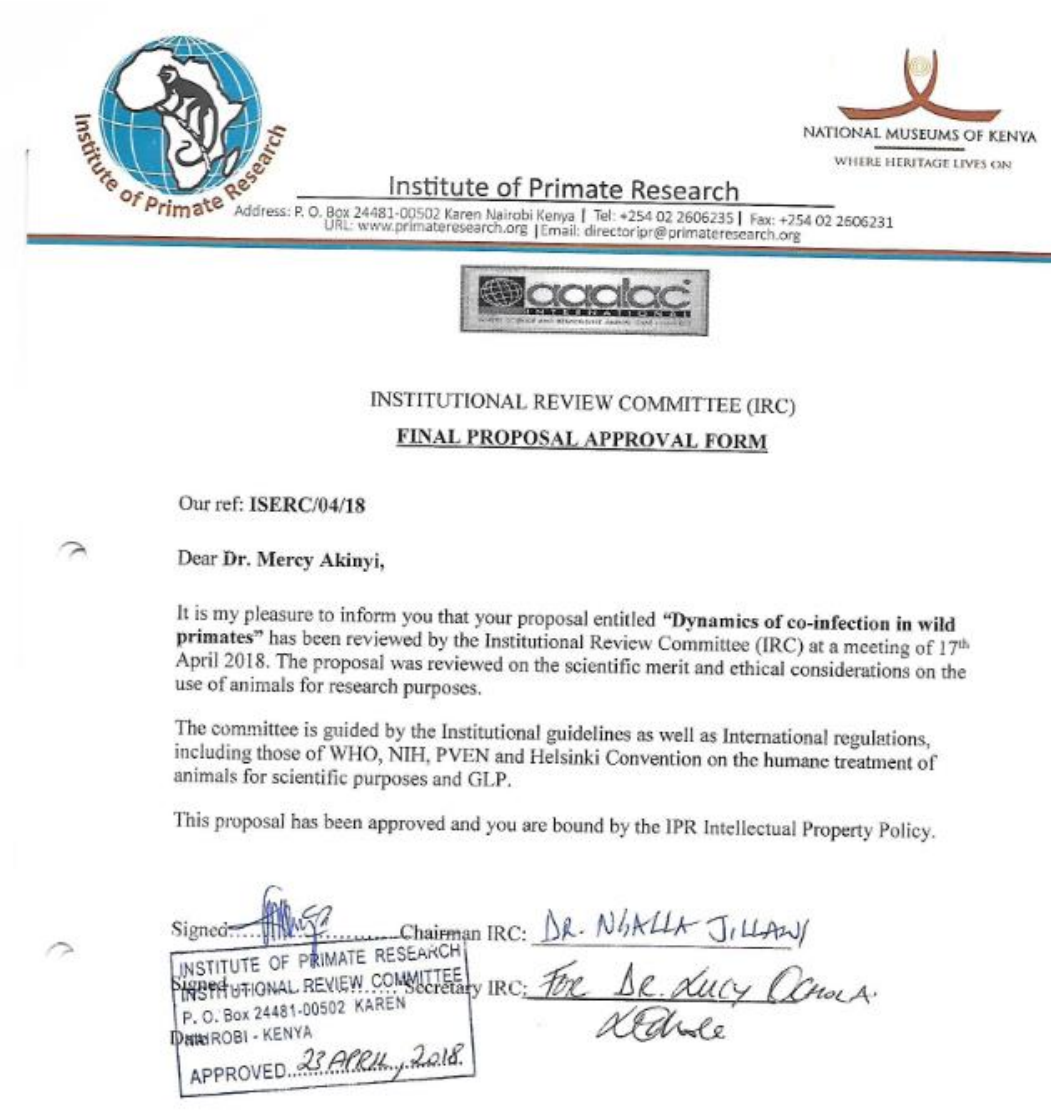
Appendix 1: Preparation of sheathers' sugar solution

Heat tap water to near boiling. Add 454g of granulated sugar to 355ml of the hot water and stir until it is dissolved. Allow the mixture to cool at room temperature. Measure the specific gravity after the solution cools (~1.27) and adjust accordingly by adding water or sugar. Add 2ml of 37% formaldehyde to prevent mold growth.

Appendix 2: Preparation of 1L of 10% formalin (neutral buffered formaldehyde)

Mix 100ml of 37-47 % formaldehyde to 900ml of distilled water. To adjust the pH, add 6.5g of sodium phosphate dibasic and 4g of sodium phosphate monobasic to the mixture.

Appendix 3: NHPS sampling permit



Institute of Primate Research

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NATIONAL MUSEUMS OF KENYA
WHERE HERITAGE LIVES ON

INSTITUTIONAL REVIEW COMMITTEE (IRC)

FINAL PROPOSAL APPROVAL FORM

Our ref: **ISERC/04/18**

Dear **Dr. Mercy Akinyi,**

It is my pleasure to inform you that your proposal entitled **"Dynamics of co-infection in wild primates"** has been reviewed by the Institutional Review Committee (IRC) at a meeting of 17th April 2018. 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 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.

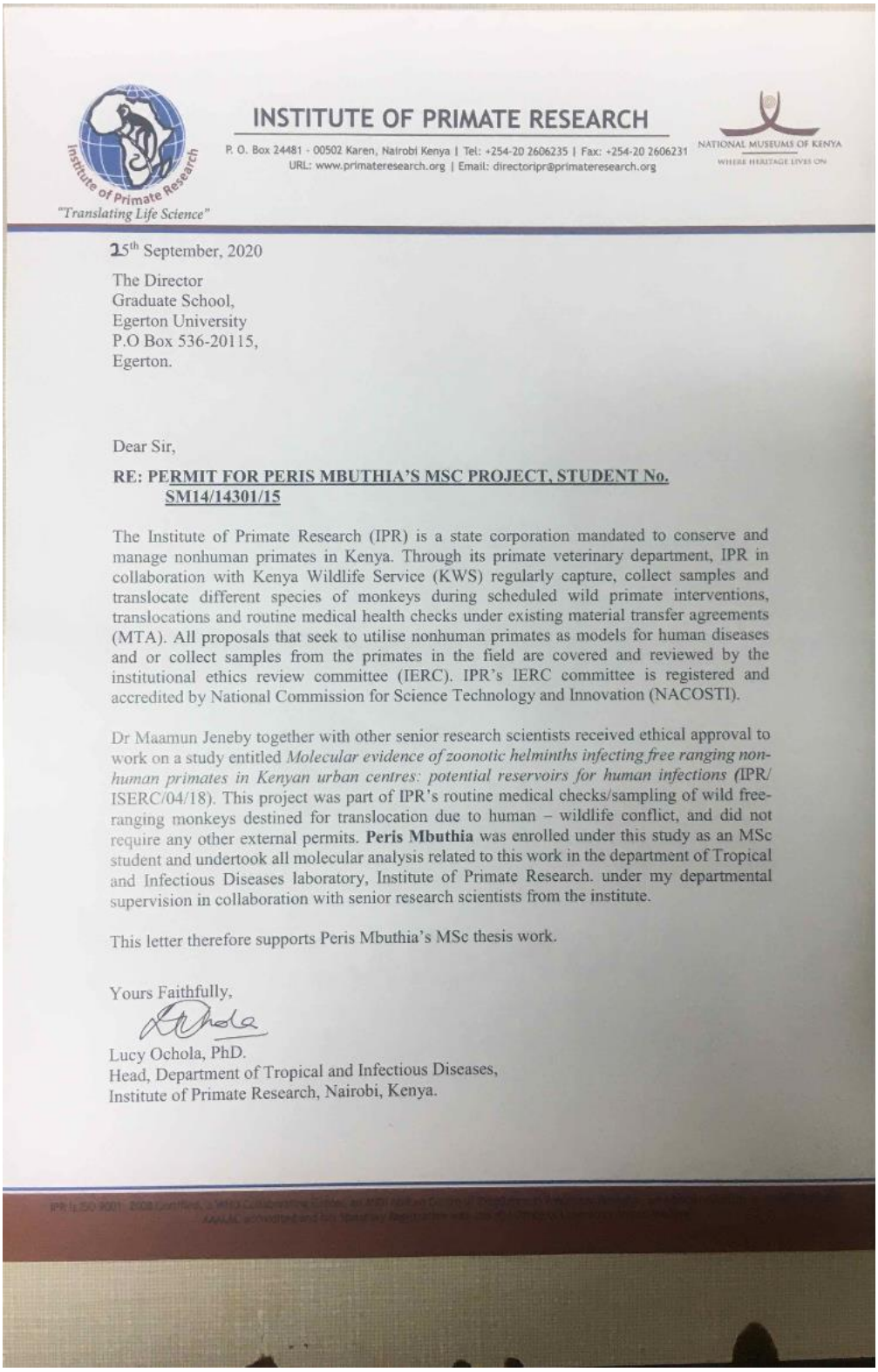
Signed.....Chairman IRC: DR. NJALLA JILLANI

Signed..... Secretary IRC: For Dr. Lucy Ochola

Date

INSTITUTE OF PRIMATE RESEARCH
INSTITUTIONAL REVIEW COMMITTEE
P. O. Box 24481-00502 KAREN
NAIROBI - KENYA
APPROVED 23 APRIL 2018

Appendix 4: Support letter (NACOSTI)



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NATIONAL MUSEUMS OF KENYA
WHERE HERITAGE LIVES ON

"Translating Life Science"

25th September, 2020

The Director
Graduate School,
Egerton University
P.O Box 536-20115,
Egerton.

Dear Sir,

RE: PERMIT FOR PERIS MBUTHIA'S MSC PROJECT, STUDENT No. SM14/14301/15

The Institute of Primate Research (IPR) is a state corporation mandated to conserve and manage nonhuman primates in Kenya. Through its primate veterinary department, IPR in collaboration with Kenya Wildlife Service (KWS) regularly capture, collect samples and translocate different species of monkeys during scheduled wild primate interventions, translocations and routine medical health checks under existing material transfer agreements (MTA). All proposals that seek to utilise nonhuman primates as models for human diseases and or collect samples from the primates in the field are covered and reviewed by the institutional ethics review committee (IERC). IPR's IERC committee is registered and accredited by National Commission for Science Technology and Innovation (NACOSTI).

Dr Maamun Jeneby together with other senior research scientists received ethical approval to work on a study entitled *Molecular evidence of zoonotic helminths infecting free ranging non-human primates in Kenyan urban centres: potential reservoirs for human infections* (IPR/ ISERC/04/18). This project was part of IPR's routine medical checks/sampling of wild free-ranging monkeys destined for translocation due to human – wildlife conflict, and did not require any other external permits. **Peris Mbuthia** was enrolled under this study as an MSc student and undertook all molecular analysis related to this work in the department of Tropical and Infectious Diseases laboratory, Institute of Primate Research. under my departmental supervision in collaboration with senior research scientists from the institute.

This letter therefore supports Peris Mbuthia's MSc thesis work.

Yours Faithfully,

Lucy Ochola, PhD.
Head, Department of Tropical and Infectious Diseases,
Institute of Primate Research, Nairobi, Kenya.

Appendix 5: Supplementary data

Table S1. Infection of non-human primates (NHPs) with soil-transmitted helminths (STH) based on gender and age groups. The number infected are shown in the square bracket as [Juvenile; Sub-adult; Adult] separated by slash sign (/) representing males and females respectively.

NHPs	Soil Transmitted Helminth (SHT)				
	Trichuris sp	*Enterobius* sp	*Ascarid* sp	*Strongyloides* sp	*Oesophagostomum* sp
AGM	[2:9:7]/[2:1:3]	[0:0:0]/[0:0:0]	[1:0:2]/[0:0:2]	[0:4:5]/[2:2:3]	[1:8:4]/[2:1:0]
Baboon	[3:3:5]/[0:3:7]	[0:1:1]/[0:0:0]	[0:0:0]/[0:0:0]	[1:2:3]/[0:3:7]	[1:1:3]/[0:1:5]
Blue Monkey	[0:0:1]/[0:0:1]	[0:0:0]/[0:0:0]	[0:0:0]/[0:0:0]	[0:0:0]/[0:0:3]	[0:1:0]/[0:0:2]
Red tailed monkey	[1:1:1]/[0:2:2]	[0:0:1]/[0:0:2]	[0:0:0]/[0:0:0]	[1:3:0]/[0:1:0]	[0:1:1]/[0:0:1]
TOTAL	[6:13:14]/[2:6:13]	[0:1:2]/ [0:0:2]	[1:0:2]/ [0:0:2]	[2:9:8]/[2:6:13]	[2:11:8]/[2:2:8]

Appendix 6: Publication

Potentially zoonotic gastrointestinal nematodes co-infecting free ranging non-human primates in Kenyan urban centres

Peris Mbuthia, Edwin Murungi, Vincent Owino, Mercy Akinyi, Gillian Eastwood, Richard Nyamota, Isaac Lekolool, Maamun Jeneby

doi: https://doi.org/10.1101/2020.08.19.254714

Abstract

Background Natural infections with soil transmitted nematodes occur in non-human primates (NHPs) and have the potential to cross primate-species boundaries and cause diseases of significant public health concern. Despite their presence in most urban centres in Kenya, comprehensive studies on their gastrointestinal parasites are scant.

Objective Conduct a cross-sectional survey to identify zoonotic nematodes in free-ranging NHPs found within four selected urban and peri-urban centres in Kenya.

Methods A total of 86 NHPs: 41 African green monkeys [AGM] (*Chlorocebus aethiops*), 30 olive baboons (*Papio anubis*), 5 blue monkeys (*Cercopithecus mitis stuhlmanni*) and 10 red tailed monkeys (*Cercopithecus ascanius*) were sampled once *in situ* and released back to their habitat. Microscopy was used to identify nematodes egg and larvae stages in the samples. Subsequently, PCR coupled with high-resolution melting (PCR-HRM) analysis and sequencing were used to identify nodule worms.

Results NHPs inhabiting densely populated urban environs in Kenya were found infected with a rich diversity of nematodes including three potentially zoonotic nematodes including *Oesophagostomum stephanostomum, Oesophagostomum bifurcum* and *Trichostrongylus colubriformis* and co-infections were common.

Conclusion Phylogenetic analysis showed that *O. stephanostomum* from red tailed and blue monkeys have a close evolutionary relatedness to human isolates suggesting the zoonotic potential of this parasite. Moreover, we also report the first natural co-infection of *O. bifurcum* and *O. stephanostomum* in free-ranging AGMs.

Competing Interest Statement

The authors have declared no competing interest.