UNIVERSITY OF NAIROBI

PREVALENCE OF GASTRO-INTESTINAL HELMINTHS IN AFRICAN ELEPHANTS (*Loxodonta africana africana*) FROM TSAVO AND LAIKIPIA-SAMBURU ECOSYSTEMS, KENYA

BY

SOPHIA KEMUNTO MOGAKA, BSc.

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DECLARATION

I declare that this thesis is my original work and it has not been presented wholly or in part for any award in any other institution

Student:

Signature: …… Date: 26th JULY 2023……

Sophia Kemunto Mogaka

Supervisors:

This thesis has been submitted with our approval as supervisors

Signature: …… Date: 25/7/2023……

Dr. Wilkinson Mutahi

Department of Biology,

University of Nairobi, Nairobi, Kenya

Signature: …… Date: 26th JULY 2023

Dr. Evans Mwangi

Department of Biology,
University of Nairobi, Nairobi, Kenya

DEDICATION

I dedicate this work to my loving parents, James Mogaka and Alice Birika Mogaka, my siblings, Ann, Joy and David, to my husband Fredrick and my daughter Brea, for encouraging and walking with me every step of the way

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ABSTRACT

African elephants host high numbers of helminths within their digestive tracts without them developing symptoms of disease. This has been attributed to a well-developed parasite host equilibrium. However, in times of stress, these high numbers of helminth within the gut pose a serious threat to the survival of the elephants, especially young ones. They do this by abrading the epithelial lining of the intestines, to gain access to the rich microvasculature, and unrestricted access to host ingested nutrients. Some of the helminths identified which affect elephants including *Protofasciola sp* and *Grammocephalus sp*, contribute to nutrition deprivation and serious damage to gut mucosa, during stressful conditions. This study sought to compare helminth infection patterns in elephant populations from Tsavo East National Park (TENP) and Laikipia-Samburu Ecosystem (LSE) through coprological evaluations. Helminth eggs and adult worms were identified and classified into genera using morphological and morphometric characteristics. General Linear Model (GLM), ANOVA and Chi squared test of independence were used to test for significant differences in variations of infection patterns observed, and to identify the effect of sex, age and location as risk factors in elephant populations with regard to helminth infections. Analyses in this study revealed that elephant populations from TENP and LSE have high prevalence rates for helminth infections. The populations are infected by nematodes, mostly belonging to the strongyle families; (*Quilonia sp, Murshidia sp, Grammocephalus sp)* and a few trematode species(*Fasciola hepatica, Protofasciola robusta*). Overall prevalence rates between TENP (95.62%) and LSE (98.53%) were not significantly different (χ^2 =2.03, p= 0.15). Prevalence of nematode infections was 97.1% which is relatively high while trematode prevalence was lower at 32.6% (χ^2 = 248.84, p<0.001). There were no significant differences in prevalence levels observed across ages; TENP (χ^2 = 1.54, p= 0.46) and LSE (χ^2 = 1.75, p=0.42). Family social group in LSE exhibited (100 %) prevalence rates while the male social group (93.3%), (χ^2 = 4.72, p= 0.007) while there was no significant differences observed in the elephant social groups in TENP (93.9% for male social group and 97.2% for family social group) (χ^2 = 0.93, p=0.335). Using Generalized Linear Model, the results indicated that age alone (p= 0.375 CI 95%) and location alone (p= 0.620, CI 95%) had no significant effect on the observed mean worm burdens as indicated by eggs per gram (EPG). Sex had a significant effect on the observed mean worm burden (p= 0.016, CI 95%), with females exhibiting higher EPGs than males. The interaction between age

and location (p<0.0005, CI 95%) and age and sex (p= 0.028, CI 95%) did have significant effect on mean EPGs observed. This information should assist wildlife authorities in developing appropriate, evidence-based health monitoring and translocation protocols for proper management of the African elephant species to ensure their sustainability.

CHAPTER ONE: INTRODUCTION

Background

The African elephant's large size, generalist feeding, foraging and high mobility, makes them hosts for a diverse species of gastro-intestinal (GI) parasites. Previous studies by Condy 1974; Obanda *et al.,* 2011 and Baines *et al.,* 2015, have recorded GI helminths in African elephants and evidenced the catastrophic effects that helminths may inflict upon their host. Some of the effects highlighted in these studies include; pathological lesions, tissue hemorrhages, necrosis and even death (Condy 1974; Vitovec *et al* 1984; Obanda *et al* 2011). Helminth infections are also known to suppress hosts immunity, stagnate their growth and decrease reproduction (Parker *et al* 2019). According to Vander Waal *et* al., (2014), GI helminths play a role in regulating wildlife populations, because infections can have detrimental health effects and may even contribute to fatalities, especially in the young.

Despite reporting of heavy helminth-parasite burdens, elephants do not exhibit clinical symptoms of infection, as is the case in most free-ranging hosts. Elsheikha and Obanda (2010) hypothesized a co-evolution of host and parasites, where disease is maintained at subclinical levels. This was described by Fowler and Mikota (2006) as a situation where, since parasitism has been in existence for millions of years, in one form or another, parasites form part of an animals' ecologic system. At optimal conditions, in their natural environment, hosts and parasites establish an equilibrium and coexist. This is because helminths produce immune-evasion molecules which neutralize immune pathways, maintaining a balance between worm expulsions and minimizing virulence. However, clinical signs of disease may begin to show upon destabilizing of the parasite- host equilibrium. Some of the factors that can destabilize this balance include; co-infections, pregnancy and lactation, adverse changes in climate; among others. These factors either directly/indirectly affect the elephant's nutritive state or its' immune response to effectively fight off the helminths, leading to development of disease. This was documented in the Laikipia-Samburu Ecosystem (LSE) in the drought of 2009 where 38 young elephants aged between 5-8 years died (Obanda *et al.,* 2011). Upon necropsy, 11 carcasses revealed pathological lesions and hemorrhages that were linked to parasitism, with identification of the nematode *Grammocephalus clathratus* and the trematode *Protofasciola robusta* and a number of unidentified adult worms in the gut.

There are some gaps in information available on GI helminths affecting elephants, the most important being the abundance and diversity of infecting species across various elephant populations in Kenya. There is need to understand GI helminth infection dynamics across various elephant habitats in order to provide a baseline for health monitoring of elephants. This will enable wildlife managers to plan for, and execute timely interventions for elephant populations that are at risk, complementing conservation efforts.

1.1 Statement of the problem

Following decline of African elephant populations over several decades due to habitat loss and poaching, *Loxodonta africana* is now listed as endangered on the "IUCN Red List of Endangered Species" in the African continent (IUCN, 2021). In order to ensure conservation efforts are effective, disease monitoring and surveillance is a critical part of wildlife management. This is because wildlife act as reservoirs for many economically important pathogens that infect domestic animals. This is especially true for helminths, which are ubiquitous and have a prevalence rate of up to 90% in cattle and elephant populations in Kenya (King'ori *et al.,* 2020 and VanderWaal *et al.,* 2014). The high prevalence of helminths in elephants may be attributed to them being very large, highly mobile animals, feeding not only on shrubs, but also on grass. This therefore means they get to graze for extensive periods in areas that are probably contaminated with helminth ova or an infective stage. The most common helminth infections in African elephants are nematode infections followed by trematode infections. Intestinal coccidian infections in elephants are common, but have not been associated with any clinical symptoms (Baines *et al.,* 2015).

In cases of severe drought, lack of adequate food and water coupled with helminth infestations has led to deterioration of body condition of young elephants, leading to emaciation, exhaustion and sometimes death (Obanda *et al.,* 2011). Studies have recorded the detrimental effects of nematode and trematodes in African elephants, especially when under stress. The elephant hookworm, *Grammocephalus clathratus* has been associated with pathological lesions and ulcerations in intestinal mucosa while the intestinal fluke *Protofasciola robusta* is linked to tissue damage, haemorrhage and even death, according to Obanda *et al.,* 2011.

All this evidence suggests that helminths play an important role in regulating wildlife populations as evidenced in Laikipia-Samburu Ecosystem (LSE) during the drought of 2009, where young

elephants died (Obanda *et al.*, 2011). They do so by affecting the elephant's ability to withstand ecological stress. This can lead to frustration of conservation efforts when a large number of elephants, especially young ones, fail to reach maturity. There is therefore need to understand the diversity and intensity of helminth infections within the various elephant populations, in order to ensure the posterity of elephants in Kenya.

Tsavo and Laikipia-Samburu ecosystems have the highest elephant populations in the country, and a comparative analysis would give a general view of GI helminth burden and species diversity in Kenyan elephants.

1.2 Justification and significance

Population management strategies for problematic animals to mitigate human-animal- conflict, elephants included, and issues of habitat loss are on the rise. One of the most common and effective strategies of dealing with problematic animals is translocation. This involves movement of animals from their resident area, to a new location. Elephants are also known to cover wide ranges, in search of food, water and other resources. During this migrations/movements, they pick up helminths from different locations and introduce them to their destinations. Health monitoring of translocated and migratory herds is crucial as they tend to harbor various helminth parasites from where they are from. These helminths are in turn introduced to the new environment, increasing the chances of infecting unsuspecting resident herds. There is therefore need to build on the body of knowledge of helminth burdens and distribution across elephant habitats to identify potential risks. Understanding helminth species diversity and abundance across different elephant populations would provide crucial information needed for health monitoring activities.

At present, information on helminth biodiversity in African elephants, is scarce and mostly 'outdated'. Since helminths form a significant part of the elephant's ecosystem, understanding the biology of infecting species presence, would also give more insight into elephant biology. This would also contribute to the body of knowledge of the various helminth species in African elephants for parasitologists and taxonomists. It is also important to note that the African elephant species is a rallying point for conservation, All the efforts put in place to conserve them will also benefit other wildlife species that inhabit and share the same ecosystems with them.

1.3 Research objectives

1.3.1 General objective

To compare occurrence of helminth parasites in elephants from Tsavo and Laikipia- Samburu Ecosystems and their potential influence to the conservation of the species.

1.3.2 Specific objectives

1. To survey helminth parasites occurring in elephants from Tsavo and Laikipia- Samburu ecosystems.
2. To compare helminth parasite prevalence in elephants from Tsavo and Laikipia-Samburu ecosystems.
3. To assess the effect of sex, age and location on helminth parasite infection intensity in eggs per gram (EPG), in elephants from Tsavo and Laikipia-Samburu ecosystems

1.4 Research hypotheses

1.4.1 Null hypothesis

There is no significant difference in helminth parasites occurring in elephant from Tsavo and Laikipia-Samburu ecosystems.

1.4.2 Alternative hypothesis

There is significant difference in helminth parasites occurring in elephants from Tsavo and Laikipia-Samburu ecosystems.

1.4.3 Assumption made in this study

This study assumes that wild elephant populations in Kenya are randomly distributed in the two ecosystems studied, and are infested with helminths. The levels of infestations may differ from population to population.

CHAPTER TWO: LITERATURE REVIEW

2.1 Role of Elephants in our ecosystems

Elephants in Kenya and Africa at large are a species whose conservation is of critical importance to governments and other relevant non-governmental organizations worldwide. Rising concern in African elephant conservation was fueled by the dramatic reduction in their numbers over the last century, due to poaching and ivory trade. In Kenya the decline in numbers, was from approximately 167,000 in 1973 to 20,000 in 1990 (Litoroh *et al.,* 2012).

The role of elephants in our ecosystems cannot be downplayed. They are a flagship species making them a rallying point for conservation efforts. They are also considered to be keystone species, having an effect on other animal species around them, for example, elephants are known to dig the ground for water during dry seasons. The pockets of water found are left for other species once they have drunk thus sustaining them. They also contribute to a large part of our economy through tourism and therefore their importance in our ecosystems and the nation cannot be overlooked. Furthermore, elephants can modify the plant structure of their environments, being able to transform bush areas by felling trees, making them an architect species (Litoroh *et al.,* 2012; Robert *et al.,* 2017).

All these make elephants an umbrella species, meaning that, conservation and protection of elephants leads to subsequent conservation and protection of other wildlife species that they co-exist with (Litoroh *et al.,* 2012). Their maintenance therefore and sustainability should be given some level of priority when it comes to matters of conservation. Elsheikha (2010), suggests that their continued existence will sustain both ecological integrity and biodiversity in the habitat in which they live.

2.1.1 Distribution of African elephants

The African savannah elephant, *Loxodonta africana africana* has been found to be genetically different from the African forest elephant, *Loxodonta africana cyclotis* (Elsheikha and Obanda, 2010; Kinsella *et al.,* 2004). However, knowledge on areas of occurrence of each sub-species and presence of unknown number of hybrid populations is lacking. Because of these uncertainties, savanna and forest elephants are thus grouped according to location; savanna elephants are found

predominantly in Eastern and Southern Africa, while forest elephants are found mainly in the Congo Basin of Central Africa and West Africa (Thouless *et al.,* 2016).

African elephants are very mobile animals, occupying a range of habitats, from tropical forest swamps, arid and semi-arid savannah bushland to desert as shown in Figure 1. Their movement is dictated by resource availability; food, water and minerals or by response to disturbance within their habitat (Litoroh *et.al.* 2012; Thouless *et al* 2016)

According to the 'African elephant status report' by Thouless *et al.,* (2016), an estimated 415,428±20,111 elephants exist in Africa, based on the areas surveyed in the last 10 years. Of these, 70% are found in Southern Africa, with Botswana having the largest national population. Eastern Africa is home to 20% of the estimated elephant populations, followed by Central Africa with 6% and West Africa with the smallest regional population of 3% (Thouless *et al.,* 2016).

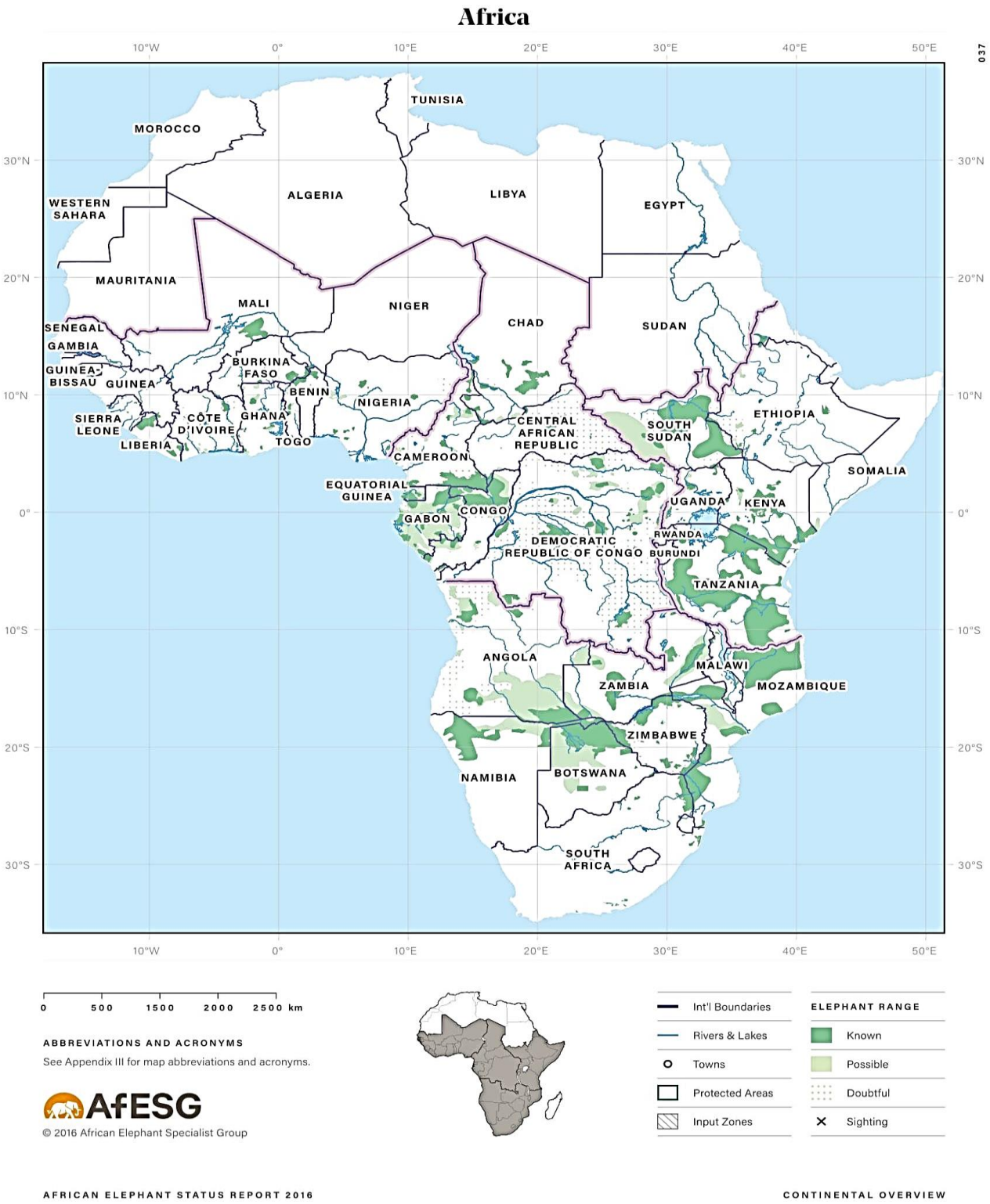


Figure 1: Elephant distribution across Africa (Thouless *et al*., 2016).

In Kenya, Tsavo National Park and adjacent areas have the largest elephant population, with an estimated 14,087±21, according to Thouless *et al.,* (2016). Laikipia- Samburu ecosystem comes in with the second largest population, with an estimated 7,347 individuals (Kenya Wildlife Service, 2017b). According to the animal census conducted by KWS between 2016 and 2017, the following are the recorded numbers of elephant in different ecosystems; Aberdare ecosystem- 3,939; Mt Kenya Forest- 2,579; Mau Forest complex- 652 and Mwea national reserve- 125 (Kenya Wildlife Service, 2017b). The Masai Mara ecosystem is home to 2,493 elephants, (Kenya Wildlife Service, 2017c). According to Thouless *et al*., (2016), during the Great Census of 2015, there was an estimated 1,736±77 elephants in the Amboseli ecosystem; 449 individuals in the Magadi area; 55 in Mt Marsabit; 659 in the Meru ecosystem; 652 in Kerio valley and South Turkana areas and 60 in Lamu district. Arabuko Sokoke forest in the coastal region, is home to an estimated 150 elephants according to Mulwa *et al*., (2013). No systematic surveys have been carried out to determine the populations of Mt Elgon forest elephants, but according to Thouless *et al*., (2016), there is an estimated 200 individuals.

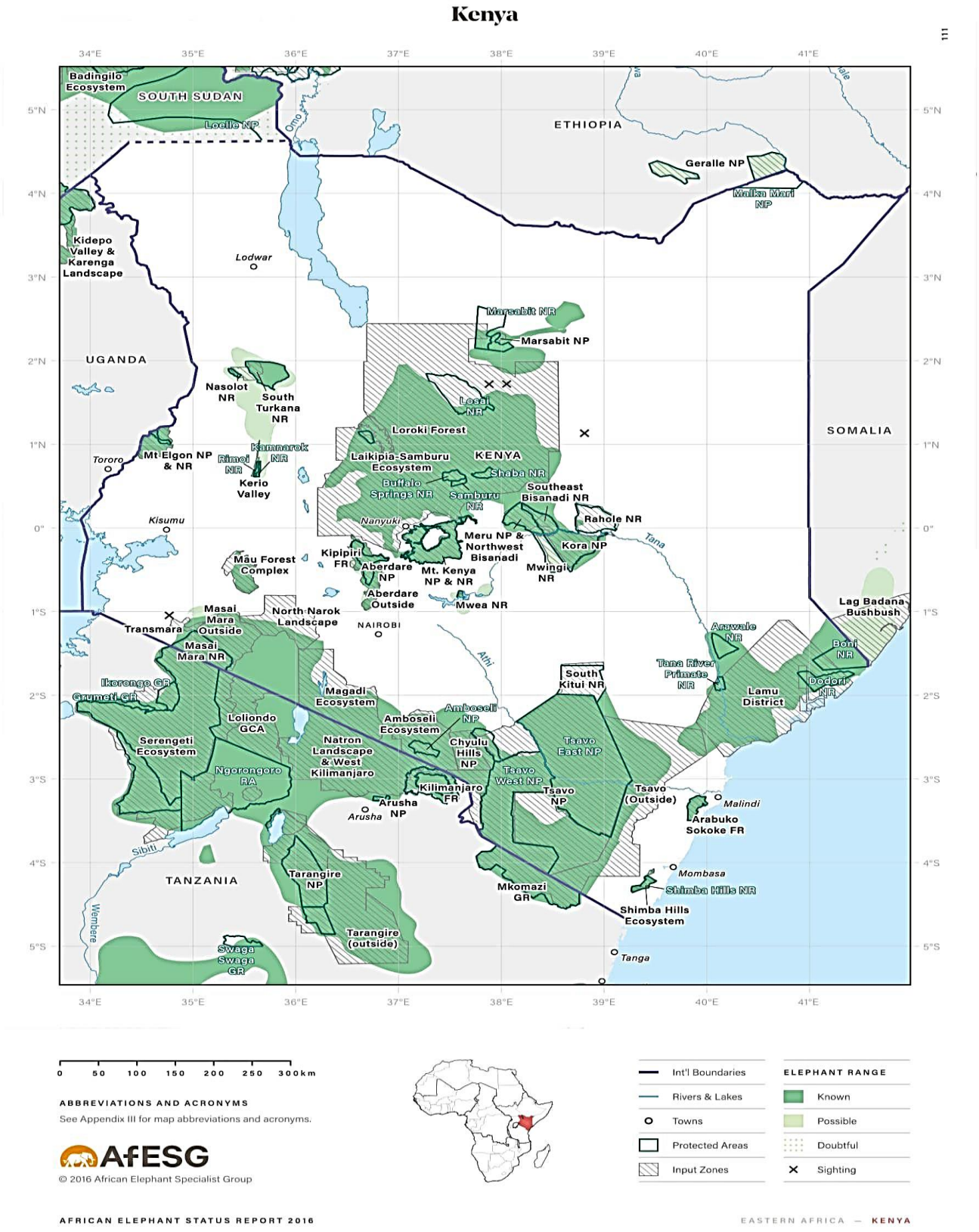


Figure 2: Map showing elephant distribution in Kenya (Thouless *et al*., 2016).

Kenyan elephants occur in both savannah and forest habitats, but all are of the savanna subspecies (*Loxodonta africana africana).* The area and range of elephant populations (Figure 2) is determined by resource distribution within the habitat, history of the use of the area and the elephants' assessment of disturbance and risk posed by people (Litoroh *et al*., 2012). Elephants are intelligent and have very good memories which enable them to be flexible and have varied responses to changes in the habitat conditions. They are able to avoid areas of high risk- where incidences of human-elephant conflict (HEC) are high to protect the herd. They are also able to recollect areas with resources, in times of drought, to sustain the herd, and this also contributes to the high mobility of these individuals.

2.2 The African elephant social structure

Elephants are mostly found in family units consisting of a group of related females and their young offspring, led by the matriarch, who is the eldest female in the group. A family group consists of eight to forty individuals in savannah elephants but forest elephants have smaller units consisting of a female and her current offspring (Fowler and Mikota, 2006). Female elephants grow and remain within the family unit. Males leave the family unit upon achieving sexual maturity at around 14 years and are known as lone males. After leaving the family unit, bachelor herds are formed, but this association does not last as long as that of the family unit. Bachelor herds consist of individuals who are approximately the same size while in family units, individuals vary in size. Males then leave the bachelor herd and wander alone, and it is bulls over 30 years old that mate with cows (Moss, 1992). Bulls only rejoin the family unit to mate, after which they leave and wander off alone.

The range of land covered by family units is relatively smaller than that covered by independent bulls due to factors like HEC and predation. Family units have calves that are more vulnerable. Bulls on the other hand, wander off to great distances, and this enables them to get the best resources even in times of resource scarcity. Different family units congregate together near swamps and water areas during times of drought and scarcity to share the available resources.

2.3 Challenges facing Kenyan elephants and their conservation

Regardless of the importance and contributions made by these mega-herbivores, the challenges they face are also tremendous. Poaching and habitat loss are the key determinants of elephant population and distribution (Litoroh *et al*., 2012). The ban on trade in ivory and the subsequent drop in ivory sales have been critical in reducing poaching incidences (Thouless *et al*., 2016). Anti-poaching campaigns by various governments and conservation authorities have seen a reduction in cases of poaching. Heavy penalties being imposed on those found guilty of poaching, possession of wildlife trophies and even roaming illegally in protected areas have proved to be a powerful deterrent.

Conversion of rangelands into agricultural and settlement schemes has led to the loss of important elephant habitats across our ecosystems (Litoroh *et al*., 2012). This is aggravated by establishment of fences and other demarcations, cutting connectivity between resources for elephants within the now fragmented habitat. This leads to formation of elephant's meta- populations. It is important to keep in mind that elephants are highly mobile animals and in a shrinking ecosystem where resources are strained, the well-maintained agricultural schemes become a risky, but available source of food, and this breeds grounds for HEC. Translocation of problematic animals is one way that governments try to prevent conflict.

Another factor to be considered is climate change, especially drought. Lack of rain means that vital resources like water and food become scarce. This then compromises the nutrient intake by elephants. This causes stress to their bodies, as they require large amounts of water, both to drink and to cool their bodies down during the day. When water and food become scarce, the survival fitness of the elephant then becomes compromised, making them extremely susceptible to infections which they would otherwise not succumb to under normal circumstances (Obanda *et al*., 2011)

These are some of the major challenges that face elephants in Kenya. The Kenyan government has however stepped up its efforts in conservation of elephants by putting up measures like banning of ivory trade and prosecuting of poachers and other wildlife criminals, translocation of elephants to reduce habitat pressures. All these have led to a steady growth in elephant numbers in Kenya (Elsheikha and Obanda, 2010).

Surveillance of infectious agents in elephant populations is critical in effective monitoring and managing of this species that is currently listed as Vulnerable (A2a) in the IUCN Redlist (IUCN, 2008). Adverse changes in climatic conditions are predicted to persist in the near future, and this poses a challenge that is difficult to overcome (Obanda *et al.*, 2011). The effect that this has on the survival fitness of the elephant and other wildlife, should not be ignored. Disease is increasingly becoming a threat to conservation of many a wildlife, including elephants. There is need to monitor patterns and trends of elephant diseases, so as to provide timely interventions, and optimize their chance of survival.

2.4 Parasitic infections in African elephants.

Parasites are known to have potentially catastrophic effects on wildlife. However, according to Elsheikha and Obanda (2010), these parasitic infections have been undermined as a formidable cause of host-species extinction even though elephants are known to have heavy parasite loads. This is due to the extensive ranges they occupy, and feeding in bulk, on both browse and grass and this influences the transmission of parasites (Litoroh *et al.*, 2012). This increases their chances of coming into contact and consuming infective helminth larvae. The neglect of intestinal helminths, as a threat to elephant conservation, may have been contributed by the fact that free-ranging elephants barely exhibit any clinical manifestations, despite having heavy parasite burdens, which have been reported from necropsies. Elsheikha and Obanda (2010), give a hypothesis that both parasites and hosts have evolved and developed a sort of equilibrium and disease only becomes apparent when the equilibrium shifts and is destabilized.

Examples of some factors that could destabilize the host-parasite equilibrium include pregnancy and lactation, adverse climatic conditions like drought, concurrent infections etc. In Kenya, elephant populations are highly fragmented into small ecosystems that are within and around national reserves and parks. The fragmentation is mostly due to habitat loss and other factors which have been mentioned above. The isolation of populations in different habitats may cause helminth diversity and helminth infection intensity patterns to differ among different elephant populations. This is because variations in environmental conditions across different habitats, has the ability to influence helminth occurrence and transmission within the hosts (Elsheikha *et al.*, 2010).

Helminth infections have been known to have devastating effects in elephants, as Baines *et al.*,

(2015), highlights. Some of these effects include pathological lesions and hemorrhages in the bile ducts, liver and intestines caused by hookworms. Elephant specific intestinal fluke *Protofasciola robusta* has been associated with intestinal tissue damage, hemorrhage and death in free-ranging African elephants as explained by Obanda *et al.,* (2007).

2.5 Parasite species infecting African elephants

Protozoan fauna in elephants have been outlined by Fowler and Mikota (2006), but have not been found to be clinically important. There are records of *Toxoplasma gondii* but no clinical manifestations of infection were recorded (Fowler and Mikota, 2006). *Trypanosoma congolense* has been recorded in elephants in Tanzania and Mozambique and *T. brucei* in elephants from Uganda (Fowler and Mikota, 2006). *Babesia sp* has also been described in the African elephant. Intestinal ciliates described include the genus *Triplumaria*, with 2 species described in the African elephant, while 9 species of the same genus were found in the Asian elephant. None of these ciliates are pathogenic (Fowler and Mikota, 2006). In Kenya, the following ciliate genera have been recorded; *Blepharocorys, Ampullacula, Didesmis, Triplumaria, Cycloposthium, Isotricha, Epidinium, Paraisotricha, Troglodytella* and many more (Obanda *et al*, 2007).

The main focus of this study is to determine the helminth species that affect savannah elephants in some of Kenya's ecosystems. A list of helminths from the phylum Platyhelminthes that have been found in African elephants have been documented by Fowler and Mikota (2006). They include those from the class **Trematoda** such as *Fasciola hepatica* and *F jacksoni*, the latter being elephant specific, *Bivitellobilharzia sp, Protofasciola sp,* and *Dicrocoelium sp*. Eggs of *Protofasciola robusta* have been recovered from elephants in LSE by King'ori *et.al* (2020) and Obanda *et.al* (2011). The same study done across Amboseli, TENP, LSE and Masaai Mara by *King'ori et.*al also recovered eggs from the trematode *Brumptia bicaudata.* Elephants get infected with trematodes, when they ingest metacercariae attached on plants. As is usual for trematodes, their lifecycle involves an intermediate snail host as demonstrated in Figure 3 (Fowler and Mikota 2006).

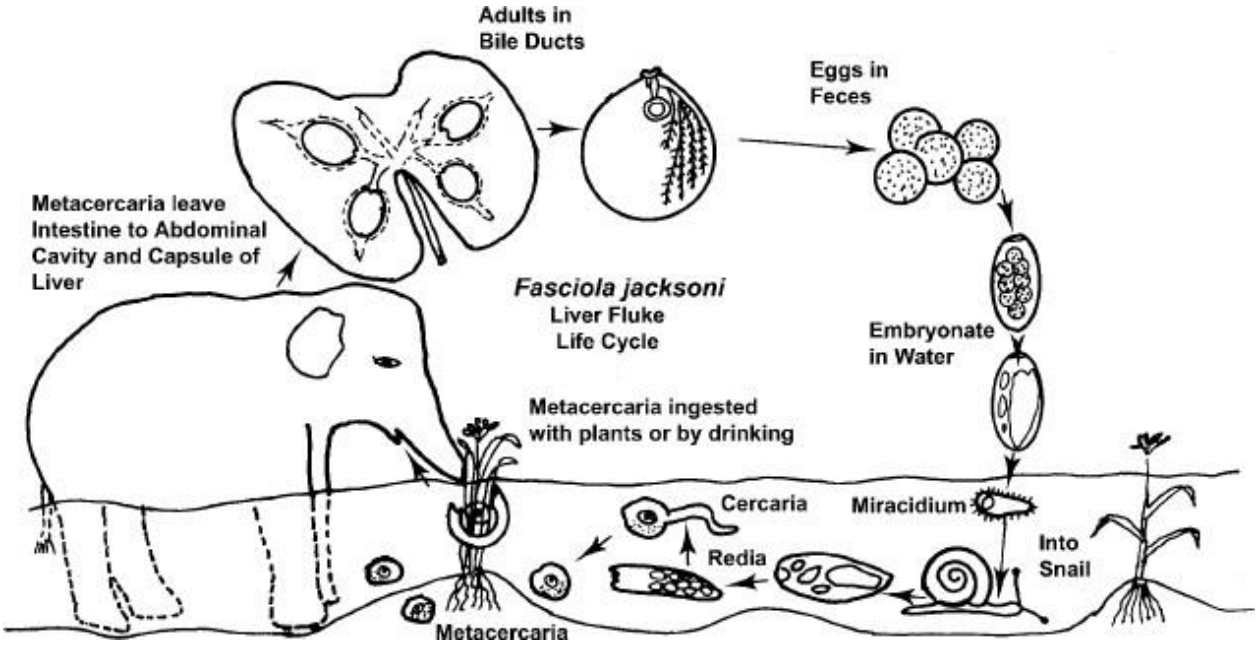


Figure 3: Life cycle of the elephant trematode Fasciola jacksoni (Fowler and Mikota 2006).

From the class **Eucestoda,** *Anoplocephala mpwapwae* is the only tapeworm species that has been identified (Fowler and Mikota 2006). In contrast, phylum **Nematoda** has numerous species. The **Order**- **Strongylida,** has the following families; the family **Strongylidae,** represented by *Chonianguin sp, Equinubria sp* and *Decrusia sp*; the family **Cyanthostomatidae,** with *Murshidia sp, Quilonia sp,* and *Khalilia sp.* Most nematodes affecting elephants belong to order Strongylida (Fowler and Mikota 2006). These undergo a direct life cycle in which elephants get infected when they ingest L3 larvae from forage as demonstrated in Figure 4. below:

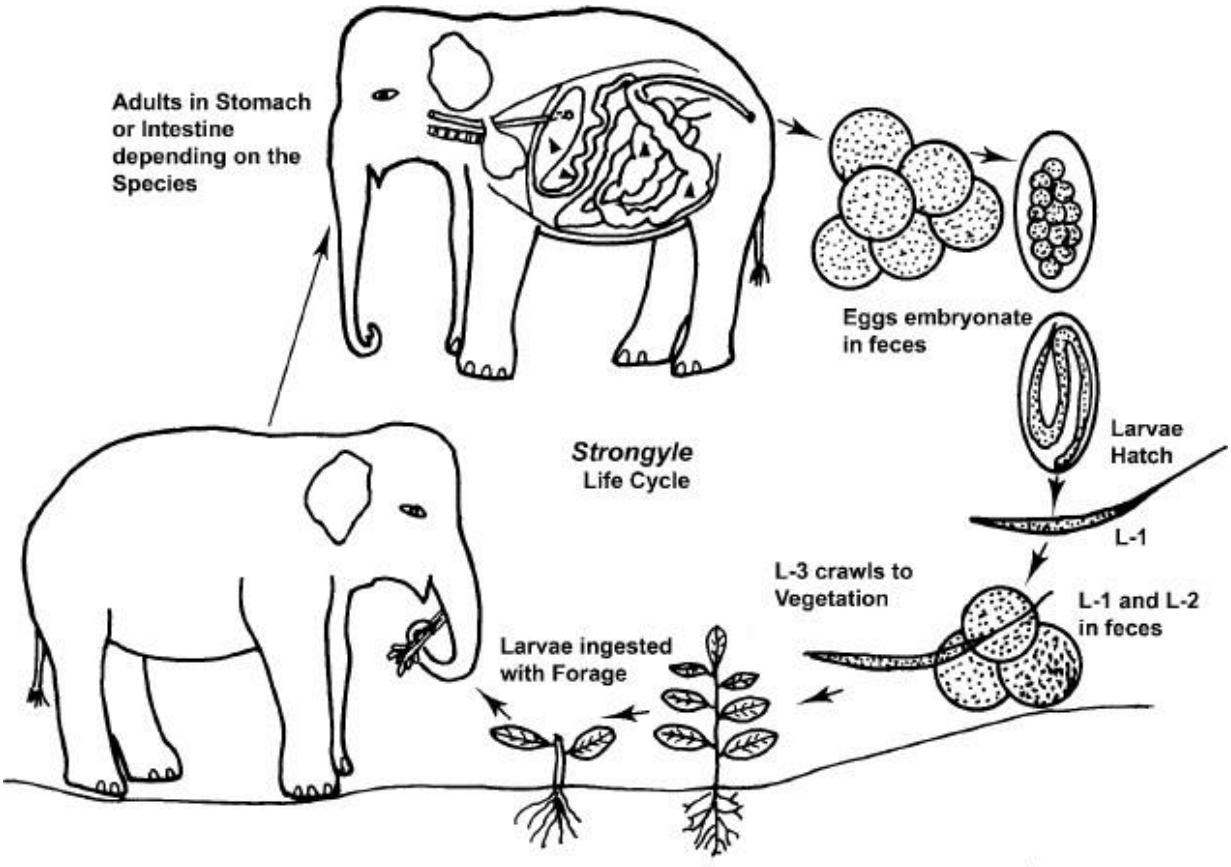


Figure 4: Typical life cycle of an elephant strongyle (Fowler and Mikota 2006).

The family **Syngamidae** contains only one species affecting elephants; *Mammomonogamus loxodantus.* The family **Atractidae** has only one genus, *Leiperenia sp*, parasitizing mammals (Fowler and Mikota 2006). In the family **Ancylostomidae,** we have examples like *Bunostomum sp* and *Gammocephalus sp* (Fowler and Mikota 2006). The former inhabit the intestines of its host, while the latter are found in the bile duct and have detrimental effects on its host (Obanda *et al*, 2011). The general lifecycle of the elephant hookworm's life is shown below in Figure 5.

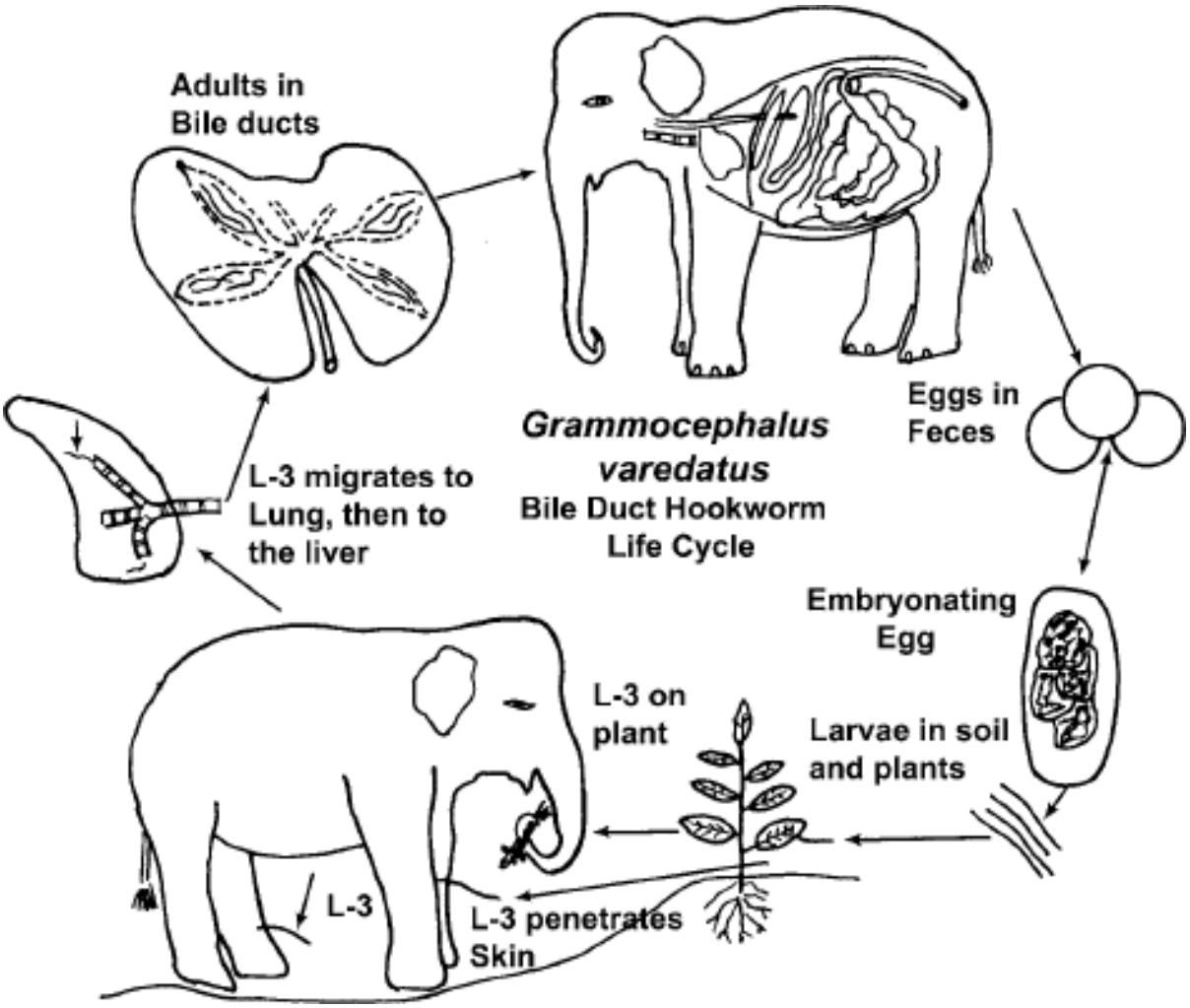


Figure 5: Life cycle of elephant hookworm Grammocephalus sp (Fowler and Mikota 2006).

Other nematodes infecting elephants belong to the **Order Spirurida,** containing the family **Acuaridae** and are represented by the genus *Parabronema* (*P. africanum, P. rhodesiense P. longispiculatum*). According to Condy, (1973) and Fowler and Mikota (2006), infections by these, have been associated with necrotic ulcers in the stomach. *Parabronema sp* are viviparous; they do not shed eggs, but instead shed larvae. L-3 larvae are deposited together with dung, and elephants get infected when they consume vegetation with L-3 larvae as demonstrated below in Figure 6. (Fowler and Mikota 2006)

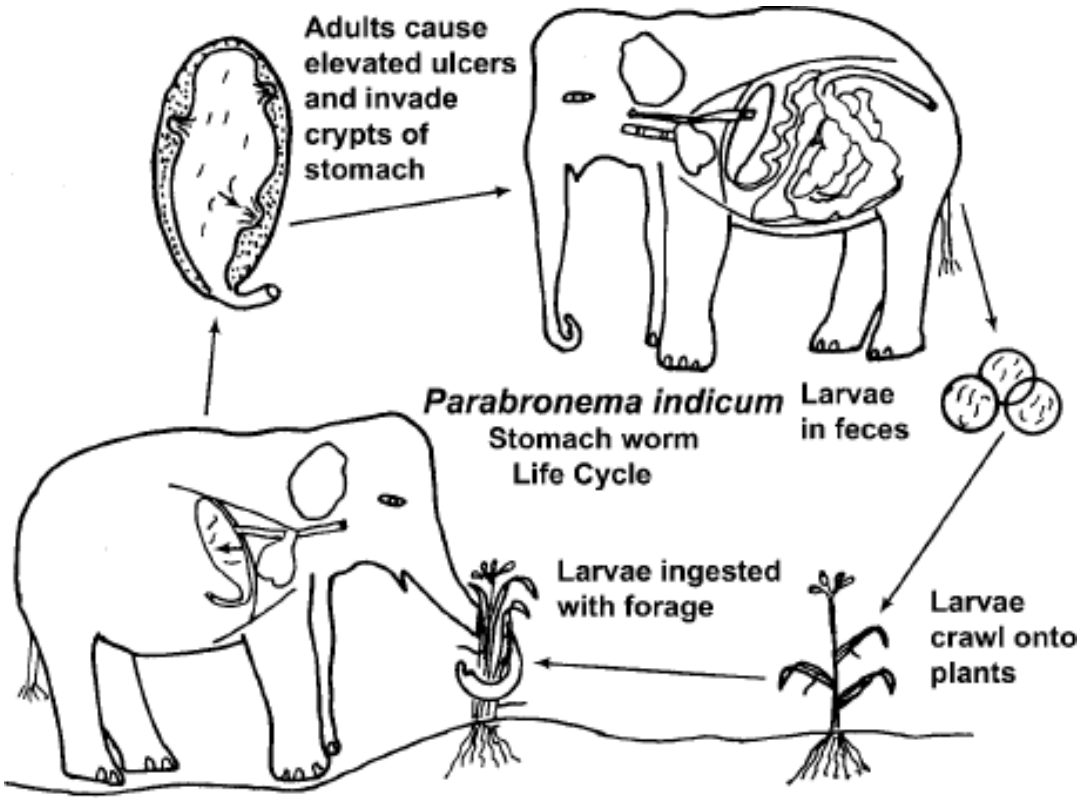


Figure 6: Life cycle of *Parabonema* sp in elephants

Filarial worms infecting elephants belong to the **Order Filariidea** and are represented by the family **Dipetalomeatidae** examples of which include *Indofilaria* (*I. pattabiramani)* and *Dipetalonema (D. gossi, D. loxodonti)*. The **Order Enoplida** contains the **family Trichinellidea** that is represented by *Trichuris* sp. Whipworm.

Helminths in African elephants have been documented in past studies from other parts of Africa, while studies on the free-ranging populations in Kenya remain vague. Translocation efforts are on the rise to reduce pressure on land and reduce conflict with wildlife and humans. Elsheikha *et al* (2010), suggests that data on parasite ecology, and more information on the different ecosystems is crucial in preliminary plans for translocation and in monitoring the health of the animals in the new habitats. This ensures successful integration of the trans-located animals. It is also important to note that since most elephants in Kenya are found in the natural environment, multiple parasitic infections are common.

2.6 Factors determining parasite richness and diversity

Both individual and environmental factors can influence in the intensity of GI parasite infections in an individual host. Parker *et al*., (2018), highlighted some individual characteristics such as age, body condition, reproductive status or sex which may influence host susceptibility to infection. Studies done on wild red deer by Albery *et al*., (2018) revealed that young and old hosts are more susceptible to infection as compared to middle- aged adults, due to the fact that their immune system is still developing while the old host's immune systems are senescing. When it comes to sex, African bull elephants were found have lower EPG counts than females and calves (Parker *et al.,* 2018; Thurber *et al.,* 2011; Mbaya *et al.,* 2013). Larger body size has also been predicted to favor parasite species richness, but actual studies have shown contradictory results, with some studies reporting positive correlation between the two variables while others found no such relationship (Morand, 2015).

Environmental factors such as season, diet and geographical range also affect infection levels in hosts. Morand (2015) states that hosts having large geographical distribution and ranges are found to have accumulated a larger burden and diversity of parasite species compared to those with less geographical coverage. This is simply explained because larger geographical range leads to more opportunities to be parasitized by several parasite species. Another angle from which the aspect of geographical range can be viewed, is in relation to the animals' utilization of space within or outside protected reserves. Community conservancies are often disrupted by human activities and this can increase stress level in animals and lower their immunity to infection. Parker *et al*., (2018), in a study on wild African elephants, found a positive correlation of strongylid infection with space use outside of protected areas.

Social life is another factor that also influences parasite transmission and infection due to close contact. Social animals have high parasite loads than non-social animals. However, a study on Grant's gazelles by Ezenwa and Worsley-Tonks (2018), showed that being social also provides some benefits that can improve the fitness cost of GI infection. Larger group sizes allocated more time to feeding, allowing them to overcome GI nematode associated emaciation. So, at the end of the day, the parasite diversity found in any wild animal species would be as a result of ecologically mediated interactions in host's life traits, from growth, survival, reproduction, and immunity.

CHAPTER THREE: MATERIALS AND METHODS.

To compare helminth parasite infections in African elephant populations in selected ecosystems in Kenya, based on location, elephant social structure, age and sex, the following work was carried out.

3.1 Study Area

Tsavo East National Park is located along the Nairobi-Mombasa highway. The park is the largest in the country and considered to be one of the largest in the world with an approximate area of 13,747sq Km. Rivers Galana, Tiva and Voi are important water features of the park, with the latter two being seasonal. Along the rivers is a narrow riverine forest and thicket dominated by *Acacia elatior, Hyphanae compressa* and *Suaeda monoica.* the north of the park is predominantly *Acacia commiphora,* while the southern part has been opened out to form open bushed grassland over the years by fires and elephants (Kenya Wildlife Service, 2010). Common shrubs here include species of *Premna, Bauhinia* and *Sericocomopsis,* and scattered trees such as *Delonix elata, Melia volkensii* and Baobab (*Adansonia digitata*. The area experiences two unpredictable rain patterns, heavier rains between April-May and light rains between November-December. The average annual rainfall ranges between 300mm-600mm (King'ori *et al*., 2019). Wildlife in this park includes the critically endangered black rhinos (*Diceros bicornis*), and hirola (*Damaliscus hunteri*), the vulnerable elephants (*Loxodont africana africana*), cheetah(*Acinonyx jubatus*) and the leopard (*Panthera pardus*). Other species in the park include African buffalo (*Syncer caffer*), lions (*Panthera leo*), several antelope species including the fringe-eared oryx(*Oryx beisa callotis*), waterbucks and lesser kudu and hundreds of bird species, and many others (Kenya Wildlife Service, 2010). Based on Kenya Wildlife Service (2017a), a census conducted between February 12th 2017 and February 21st 2017 estimates the elephant population in this ecosystem to be around 7,727 individuals.

The Laikipia-Samburu ecosystem covers approximately 25000Km^2, and is located in the central heartland of the country and is considered to be semi-arid, with an annual rainfall of around 300mm in the northern region and 700mm in the southern regions of the ecosystem. Rainfall is bimodal and falls in April-May and November-December. It is characterized by hills, plateaus and rough terrain. The ecosystem has sic major land use types: National reserves, state-protected forest reserves, communal pastoral areas, community conservancies, private ranches and settlements under

subsistence production, all sharing the scarce resources within. This resource sharing becomes even more stressful in times of drought, when resources become diminished, fueling human-elephant conflict. Wildlife found in this ecosystem include the near threatened Northern white rhino(*Celatotherium simum cottoni*) the critically endangered black rhino(*Diceros bicornis*)the endangered reticulated giraffe(*Giraffa Camelopardalis reticulata),* grevy zebra(*Equus grevyi),* the vulnerable Somali ostrich(*Struthio molybdophanes*), elephants(*Loxodont africana africana*), cheetahs and leopards; the beisa oryx(*Oryx beisa)* the gerenuk(*Litocranius walleri*), buffaloes, lions, several other antelope species, hundreds of bird species and many others. Elephant populations in this area at the time of this study were estimated at 7,166 according to the census conducted in November 19 2017 (Kenya Wildlife Service, 2017).

3.2 Sample size determination

Samples were purposively and opportunistically collected from different herds or lone males that were encountered.

Sample sizes were determined using statistical formula as given by (Thrusfield, 2007):

$$n = \frac{1.962^2 \times Pexp(1 - Pexp)}{d^2}$$

Where;

n = required sample size,

Pexp = expected prevalence (90%)

d= desired absolute precision (5%)

Thus;

$$n = \frac{1.962^2 \times 0.9(1 - 0.9)}{0.5^2} = 138$$

The above formula is based on an infinite population and yields n=138 individuals. In cases where the population is smaller, a smaller sample size can be obtained to achieve the same degree of precision using the formula below (Thrusfield, 2007):

$$n_{adj} = \frac{N \times n}{N + n}$$

Where;

n_{adj}= required sample size

N= size of the study population n= 138

Table 1: Table showing sample size calculation for both study areas.

TENP	LSE
$n_{adj} = \frac{7727 \times 138}{7727 + 138} = 135.57$	$n_{adj} = \frac{7166 \times 138}{7166 + 138} = 135.39$

With the above formula, optimal samples collected vs actual samples collected were; from Tsavo, 136 vs 137 samples, and 135 vs 136 samples from the Laikipia-Samburu Ecosystem respectively.

3.3 Sample collection

Freshly voided fecal samples were collected opportunistically, between 6am and 6pm. Following the methods of Baines *et al*., (2015), elephant herds or lone males once spotted, were observed until they had defecated and moved off to a safe distance. Sample collection involved use of fecal pots, gloves, wooden splints and 10% formalin as a preservative. Samples were collected purposively and opportunistically, mainly at watering points and each animal was only sampled once. To assist with this, the use of field experts was also employed to enable differentiation of elephant herds and lone males.

Samples would be picked from different boluses of the same animal, where available. The sample included some dung from the top of the dung bolus, from the center and from the bottom of the dung bolus. They were placed in fecal pots (50ml and 100ml), labeled and 10% formalin was added. The wooden splints were used to stir the dung sample with the 10% formalin solution to

ensure uniformity in preservation. The fecal pots were then sealed and stored. Demographic variables collected for individuals were age, gender, and family group. All samples in this study were collected between June- October 2019, before the rains, to account for seasonality when it comes to comparing results between the two locations. All samples were transported to KWS headquarters veterinary laboratory where they were analyzed for intestinal parasites.

3.4 Sample analysis

In the laboratory, samples were processed for parasitological examination. For qualitative analysis, centrifugal Sheather's sugar floatation and water sedimentation methods were used. As for quantitative analysis, McMaster egg counting technique, using Sheather's sugar solution as the floatation fluid was used. Sorting dung for identification of adult worms was also conducted.

3.4.1 Centrifugal floatation method

The principle of floatation is a concentration process based on the fact that parasite eggs are less dense than floatation solution and will thus float to the surface of the test tube or container being used, from where they can be picked for examination. The most common floatation solutions and their specific gravity include sodium chloride (SPG 1.20), zinc sulphate (SPG 1.18) and Sheather's sugar solution (SPG 1.27). Sheather's sugar solution is the floatation medium used for this analysis. This is because, compared to zinc sulphate and sodium chloride, it has the highest SPG of the three. This means that zinc sulphate and sodium chloride will not float the denser nematode eggs and taenid eggs. According to Zajac and Conboy (2012), Sheather's sugar solution does not distort eggs as rapidly as salt solutions do, making it ideal. In addition, centrifugation helps the eggs float more rapidly and increases sensitivity of fecal examinations.

Sheather's sugar solution was used for this process. The floatation solution was prepared by combining 454g of sugar (KABRAS Sugar) with 355ml of distilled water. The mixture was heated at low temperatures while stirring intermittently until all the sugar dissolved. The mixture was then left to cool before use.

Approximately 3g of fecal sample was weighed and placed in a conical flask and 12ml of water added and the mixture strained through a sieve after mixing. The filtrate was transferred to a 15ml centrifuge tube and topped up with water up to the 14ml mark and centrifuged at 1500 rpm for 10 min. The supernatant was poured off and the sediment at the bottom re-suspended, first filled half

way with Sheather's sugar solution, mixed using a wooden splint and filled to the top with the sugar solution until it forms an upper meniscus and a cover slip is gently placed on the center.

The mixture was left to stand for around 20 min to allow the cover slip to adhere to the centrifuge tube and avoid breakage. The mixture was then centrifuged at 1500 rpm for 10min after which the cover slip removed gently and placed on a glass slide for observation under the microscope (X400) (MICROTEC IS300, ISCapture.Ink). Micrographs were taken and dimensions of helminth ova measured using the ISCapture micro-imaging software version (ISCapture.Ink)

3.4.2 Sedimentation method

The process of sedimentation is to enable concentration of denser eggs that do not readily float in floatation mediums, like the eggs of trematodes and, some nematodes and cestodes. This is accomplished through a simple process involving water or using formol ether. In this case, simple water sedimentation process was used because when using formol ether technique, a lot of debris was present making it difficult to view parasite eggs. The process followed was adopted from Vanderwaal *et al,* (2014). Approximately 3g of the dung sample was weighed and mixed with 45 ml of tap water. The mixture was strained and the filtrate transferred to a 50ml centrifuge tube. The filtrate was left to sediment for around 30 minutes and the supernatant gently decanted out. The sediment was re-suspended with 45ml of tap water and left to stand for at least 10 minutes. Re-suspension and decanting is was repeated until the suspension is clear. 200ul of the sediment was then pipetted onto a glass side and covered with a coverslip for observation. Image processing followed the same as that of floatation method using MICROTEC IS300, ISCapture.Ink microscope and imaging software.

3.4.3 Mc Master Method.

To quantify the burden of helminth infestation, a modified Mc Master method adopted from Foreyt (2001) was used, using sheather's sugar as the floatation fluid. It follows the same procedure as the sugar floatation, until the point where the sediment is mixed with the Sheather's sugar solution to form an upper meniscus. No cover slip was placed at this point, instead, the mixture in the 15ml centrifuge tubes were left to stand for about one and a half hours to allow the eggs sufficient time to float to the surface. A pipette was used to transfer suspension from the surface to a McMaster slide, filling both chambers. The slide was placed on a microscope (MICROTEC IS300) and eggs

inside the chambers counted under ×10 magnification. The number of eggs counted were multiplied by 50 for to establish the estimated eggs per gram. For both quantitative and qualitative analysis, 137 dung samples from TENP and 136 dung samples from LSE were analyzed in each case. Each animal was sampled once.

3.4.4 Adult worm identification

Using egg measurements alone for identification of species present presents a few challenges. It has been noted that egg measurements for a single species varies greatly across different elephant populations (King'ori *et al.,* 2020). In other scenarios, we have eggs whose measurements are outliers and therefore don't fall in the range of measurements provided in publications. To overcome this downfall, it is important, where possible, to study larval stages and adult worm morphology to determine species present. The most assured way however for species determination is through molecular characterization, as was done by McLean *et al.,* (2012). In this case, manual sorting of dung samples collected from TENP and LSE was done to look for adult worms present in the dung. Adult worm processing followed a modified method of McLean *et al.*, (2012). Worms found were placed in clean sample bottles containing 10% formalin. The worms were later placed on a slide and cleared using glycerol. A coverslip was placed on top of the slide and the sample let to sit for 1 week to achieve clearing before observation (Obanda *et. al.,* 2011). After a week, the slide was placed on the microscope (MICROTEC IS300) for observation under X100 magnification, and images of anterior and posterior regions were recorded using ISCapture micro-imaging software version (ISCapture.Ink). Whole worms were observed using a dissecting microscope (Leica EZ4D) and images captured using the supporting software LAS EZ. To identify genera present, focus was on studying the morphology of the anterior and posterior ends, and comparing with the published works of (Anderson *et al.,* 1974; Monnig, 1925 and Van Der Westhuysen, 1938).

3.5 Data analysis.

Egg measurements obtained from the methods described above, were used to identify species by comparing them to known egg sizes. While adult worms obtained were compared with those of known genera and species, using mostly the morphologies of the anterior and posterior regions of

the worms, for classification purposes.

Floatation and sedimentation data obtained was used to calculate prevalence of infection for comparison by location, sex and age. Prevalence was calculated as follows;

$$\frac{\text{Number of infected elephants}}{\text{Total sample population}} \times 100$$

Resulting count data on presence/absence of disease for prevalence was compared based on location, social group and age. Differences observed were tested for significance using chi square test of independence at 95% CI, using the IBM SPSS version 20 software. To examine the effect of interaction of location, age, social group and sex on EPG, Generalized Linear Model (GLM), with a fitted Poisson distribution using the IBM SPSS version 20 software was used.

CHAPTER FOUR: RESULTS

Analyses conducted in this study shows that elephants from TENP and LSE are infected by a wide range of nematodes mostly strongyles, and a few trematodes. Both populations recorded high prevalence rates. Overall, prevalence rates between TENP (95.6%) and LSE (98.5%) were not significantly different (χ^2=2.03, p= 0.154), neither were they significantly different across ages; TENP (X^2 = 1.544, p= 0.462) and LSE (χ^2 = 1.752, p=0.416) as shown in Table 2 and Table 3. Family social group in LSE exhibited prevalence rates that were significantly different from the male social group, (χ^2 = 4.715, p= 0.007) while there was no significant differences observed in the elephant social groups in TENP (χ^2 = 0.93, p=0.335) as indicated in Table 2. The prevalence of nematode infections was 97.1% while that of trematodes was significantly different at 32.6% (χ^2 = 248.836, p<0.001), as shown in Table 2. The results indicated that, in general, rates of helminth infection and the egg loads (EPG), were not statistically different between elephants in TENP and in LSE, (p= 0.620, CI 95%). Sex had a significant effect on mean worm burden (p= 0.016, CI 95%), with females exhibiting higher EPGs than males. However, EPG values within each location, differed significantly on the basis of sex (p= 0.028, CI 95%) and age (p< 0.001, CI 95%).

4.1. Intestinal parasites in elephants from TENP and LSE areas.

4.1.1. Parasite survey based on egg morphology and morphometry

A total of 137 elephants from TENP and 136 from LSE were sampled for this analysis. Floatation and sedimentation analyses revealed that elephant populations from the sampled areas are infected by nematodes whose eggs were of a typical strongyle-type morphology, with a morphometric range between 55-90 µm long and 35-55 µm wide as also identified by Baines *et al.,* (2015). Genus identification was based on comparison of egg measurements taken from samples and published egg dimensions of helminths affecting African elephants (Condy, 1974; Fowler and Mikota, 2006; Van Der Westhuysen, 1938). Trematode species identified had similar lengths to those of *Fasciola hepatica* (Figure 7. a) and *Protofasciola robusta* (84-104*56-64 µm, Figure 7. b and c). The eggs of *Fasciola hepatica* were easily distinguishable from those of the other trematodes due to the absence of an operculum, which is distinct in *Protofasciola robusta* and *Brumptia bicaudata* species (Fowler and Mikota, 2006). Some nematode genera identified from eggs and their subsequent mophometry have been shown in Figure 7. They included those of *Murshidia* (70-

75*35-50 µm, Figure 7. d, e and f), *Quilonia* (80-90*40- 55 µm, Figure 7. g., h, and i), *Grammocephalus* (65-75*40-50 µm, Figure 7. j) and *Khalilia* (80-92*44-60 µm, Figure 7. k).

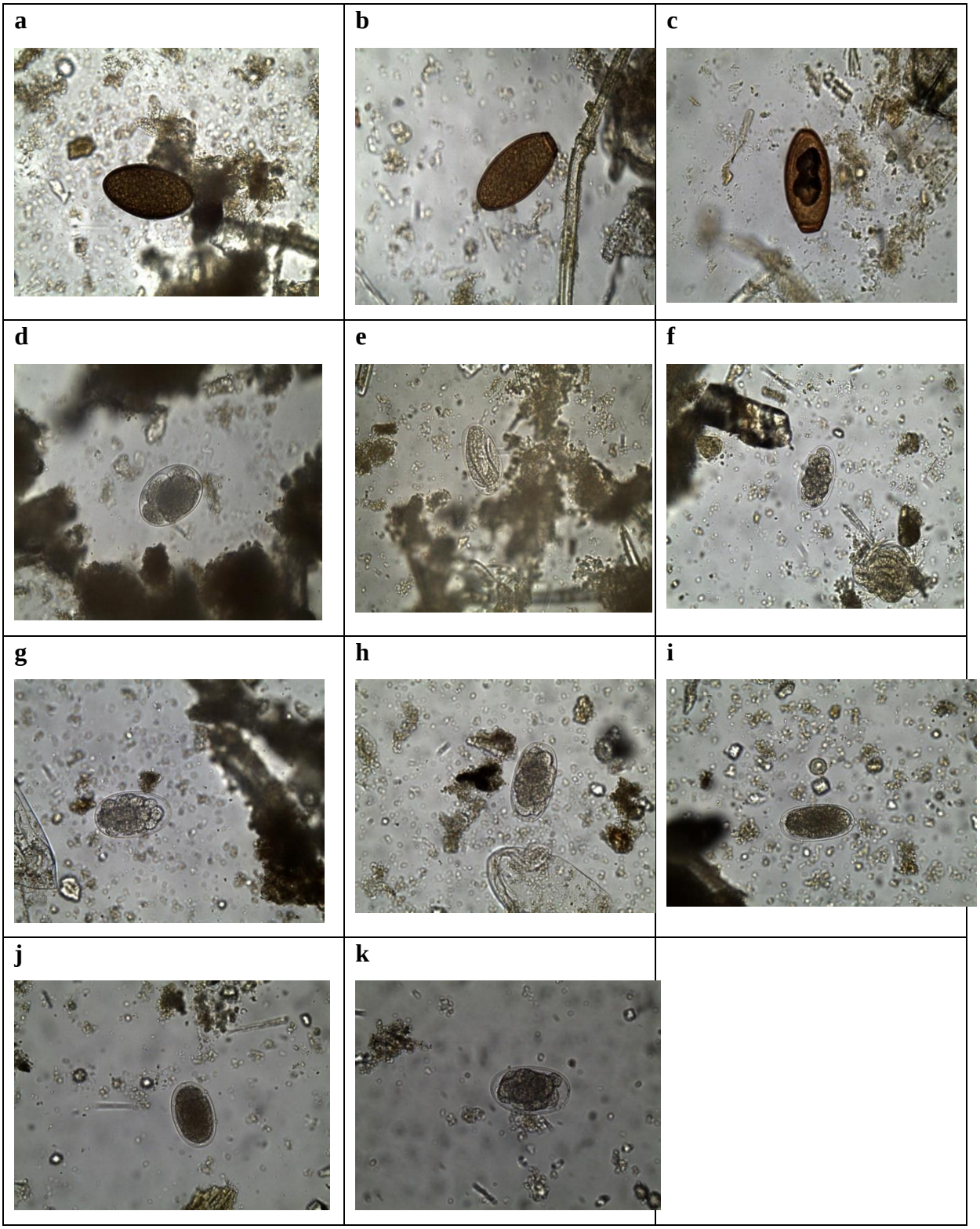
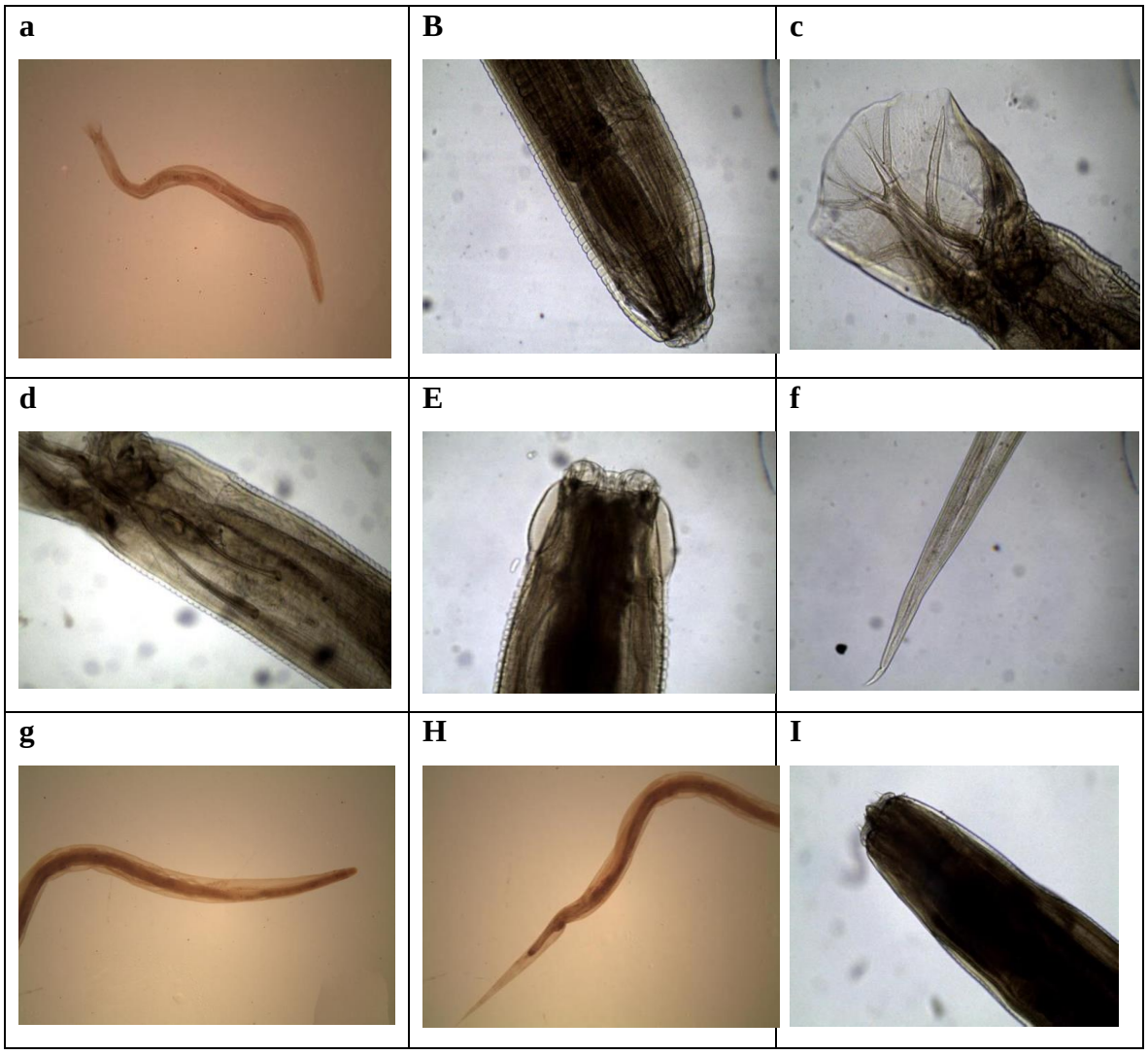


Figure 7: Photomicrographs (tiles: **a-k)** of eggs from nematodes and trematodes of 4 genera: a. Fasciola (99.47*52.42 µm), b. and c. are different sizes of Protofasciola: b. (98.39*50.28 µm), c (µm): d., e. and f. are different sizes of Murshidia d. (71.2*50.46µm) e. (72.27*39.

4.1.2. Adult worm identification

No adult worms were recovered from the TENP samples. However, from the LSE samples, 29 worms were recovered from 8 out of the 136 samples collected. Of the worms recovered, 26 were identified up to genus level. Using keys and descriptions provided by Monnig, (1925); Van Der Westhuysen, (1938) and Anderson *et al.*, (1974), infections from *Quilonia sp* (Figure images **a-f**) and *Murshidia sp* (Figure images **g-u**) were identified. The morphology of the anterior and posterior regions of the worms were found most useful for identification



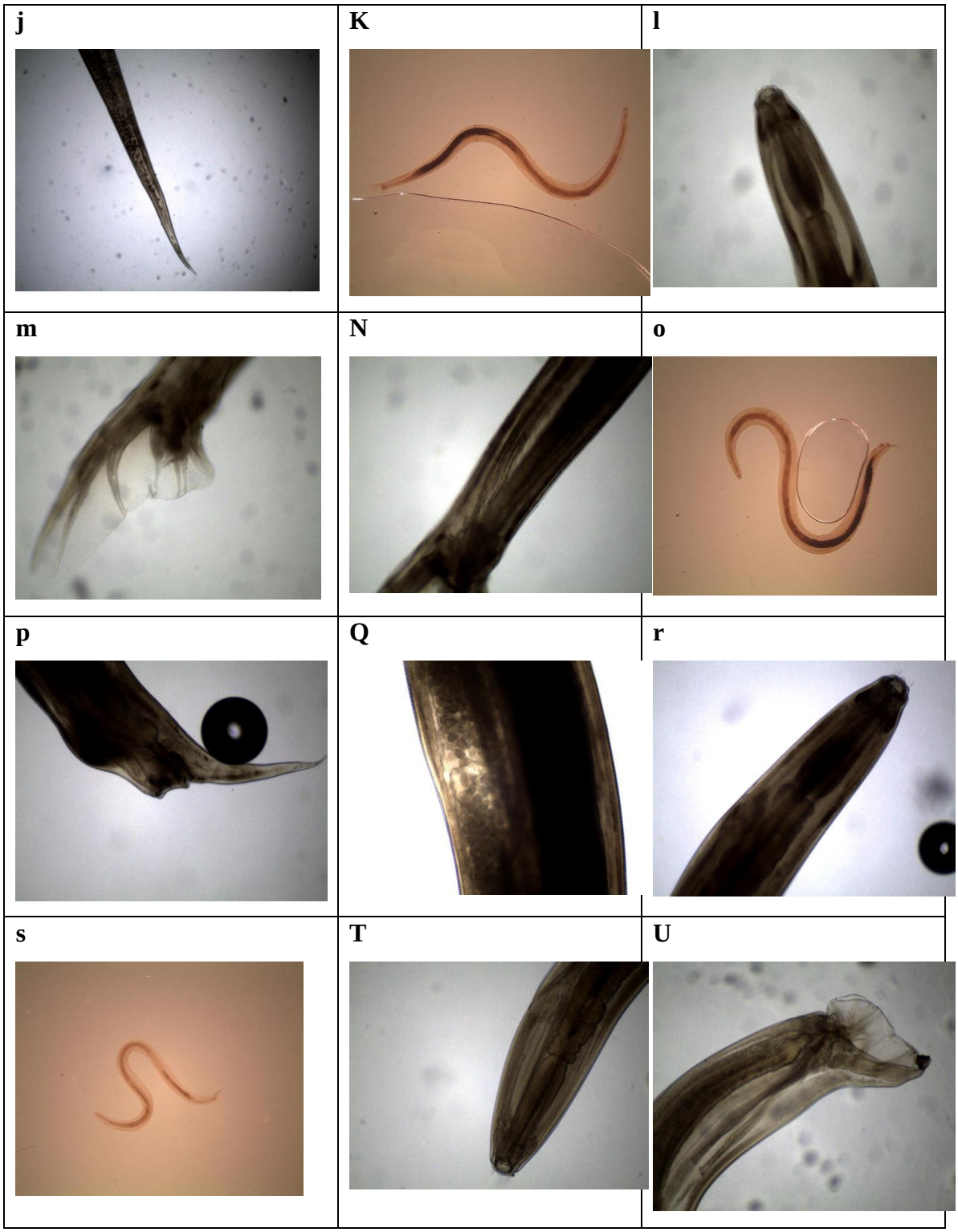


Figure 8*:* Photomicrographs (tiles: a-u) of adult nematode worms isolated from faecal samples. *Quilonia* sp. male: **a**. whole worm ×8, b. anterior/ head region, **c**. Tail showing corpulatory bursa,

d. Tail region showing spicules, *Quilonia sp*. female: **e**. anterior region/head, **f**. Tail, Murshidia sp. female: whole worm ×8: **g**. anterior, **h**. posterior, **i**. anterior region/head , **j**. tail region, Murshidia sp. male: **k**. whole worm ×8: **l**. anterior/ head region ×100, **m**. Tail region with corpulatory bursa ×100, **n**. Tail region with arrow showing spiculon ×100, Murshidia sp. female: **o**. worm ×8, **p**. Tail region ×100, **q**. eggs in gravid female , **r**. anterior/ head region , Murshidia sp. male: **s**. whole worm ×8: **t**. anterior/ head region , **u**. tail region showing corpulatory bursa and spicules ×100.

4.2. Comparison of helminth occurrence in TENP AND LSE

4.2.1. Comparison of prevalence

A total of 273 individual elephants were examined for intestinal helminths. Out of these, 137 (50.2%) individuals were from TENP and 136 (49.9%) individuals from LSE. Based on social grouping, individuals sampled from male social groups were 95 (34.8%) while those samples from family social groups were 178 (65.2%). Adult elephants sampled in this study were 160 (58.6%) of which 87 individuals were from TENP and 73 from LSE. Sub adults were 68 (24.9%), with 30 individuals from TENP and 38 from LSE. Lastly, total number of juveniles sampled were 45 (16.5%) with 20 individuals from TENP and 25 from LSE. Prevalence rate established from floatation method was 93% while that obtained from sedimentation was 97.1%. The difference in prevalence observed while using the two methods may be explained by the fact that most floatation techniques cause the walls of the eggs to collapse thus hindering identification, and not all parasite eggs will float.

Table 2: Total number of infections and prevalence rates of helminth infections recorded in elephants from TENP and LSE, estimated using sedimentation method.

		TENP	**LSE**	**TOTAL**
	Count (N)	137	136	273
Nematode infections	Number infected (n)	131	134	265
	% prevalence	**95.6**	**98.5**	**97.1**
Trematode infections	Number infected (n)	54	35	89
	% prevalence	**39.4**	**25.7**	**32.6**

There was no significant difference in prevalence rates based on location (χ^2 =2.03, p= 0.15). The prevalence of trematodes (32.6%) lower than that of nematodes (97.1%) (χ^2 = 248.84, p<0.001). Elephants from TENP (39.4%) had a trematode prevalence rate that was higher than that observed from the population in LSE (25.7%) (χ^2 =5.81, p= 0.016).

Table 3: Number of infections and prevalence rates of helminth infections based on elephant social groups, recorded in elephants from TENP and LSE, estimated using sedimentation method.

		TENP	LSE	TOTAL
Male social group	Count (N)	65	30	95
Nematode infections	Number infected (n)	61	28	89
	% prevalence	**93.9**	**93.3**	**93.7**
Trematode infections	Number infected (n)	31	5	36
	% prevalence	**47.7**	**16.7**	**37.9**
Family social group	Count (N)	72	106	178
Nematode infections	Number infected (n)	70	106	176
	% prevalence	**97.2**	**100**	**98.9**
Trematode infections	Number infected (n)	23	30	53
	% prevalence	**31.9**	**28.3**	**29.8**

There was no significant difference in observed prevalence rates in the male and family social groups of the elephant populations of TENP (χ^2 = 0.93, p=0.335). In LSE however the family social group recorded a prevalence rate (100%) that was higher than that of the male social group (93.3%) (χ^2 = 4.715, p= 0.007). There was no significant difference in trematode prevalence based on social groups from both locations: TENP, (χ^2 = 3.55, p= 0.06) and LSE, (χ^2 = 1.66, p= 0.198).

Table 4: Number of infections and prevalence rates of helminth infections based on elephant age groups, recorded in elephants from TENP and LSE, estimated using sedimentation method.

		TENP	LSE	TOTAL
Adult	Count (N)	87	73	160
Nematode infections	Number infected (n)	83	71	154
	% prevalence	**95.4**	**97.3**	**96.3**
Trematode infections	Number infected (n)	34	18	52
	% prevalence	**39.1**	**24.7**	**32.5**
Sub-adult	Count (N)	30	38	68
Nematode infections	Number infected (n)	28	38	66
	% prevalence	**93.3**	**100**	**97.1**
Trematode infections	Number infected (n)	10	10	20
	% prevalence	**33.3**	**26.3**	**29.4**
Juvenile	Count (N)	20	25	45
Nematode infections	Number infected (n)	20	25	45
	% prevalence	**100**	**100**	**100**
Trematode Infections	Number infected (n)	10	7	17
	% prevalence	**50**	**28**	**37.8**

There is no significant difference in overall helminth prevalence rate observed between adults, sub-adults and juveniles in both TENP (χ^2 = 1.54, p= 0.462) and LSE (χ^2 = 1.75, p=0.416). Trematode prevalence based on age also shows no significant differences in both TENP (χ^2 = 1.4q, p= 0.495) and LSE (χ^2= 0.12, p= 0.943).

4.3. Comparison of worm burden

Generalized Linear Modeling at 95% confidence level was used using IBM SPSS Statistics 20, to determine the effect of age sex and location on the worm burden. Based on this model, it was possible to determine the effect of location, age and sex, on the mean worm burden observed. Results obtained from showed that age alone (p= 0.375 CI 95%) and location alone (p= 0.620, CI 95%) had no significant effect on mean worm burdens observed. Sex had a significant effect on mean worm burden (p= 0.016, CI 95%), with females exhibiting higher EPGs than males.

Interaction between age and location (p< 0.001, CI 95%) and age and sex (p= 0.028, CI 95%) did have significant effect on mean EPGs observed as shown in the plots below:

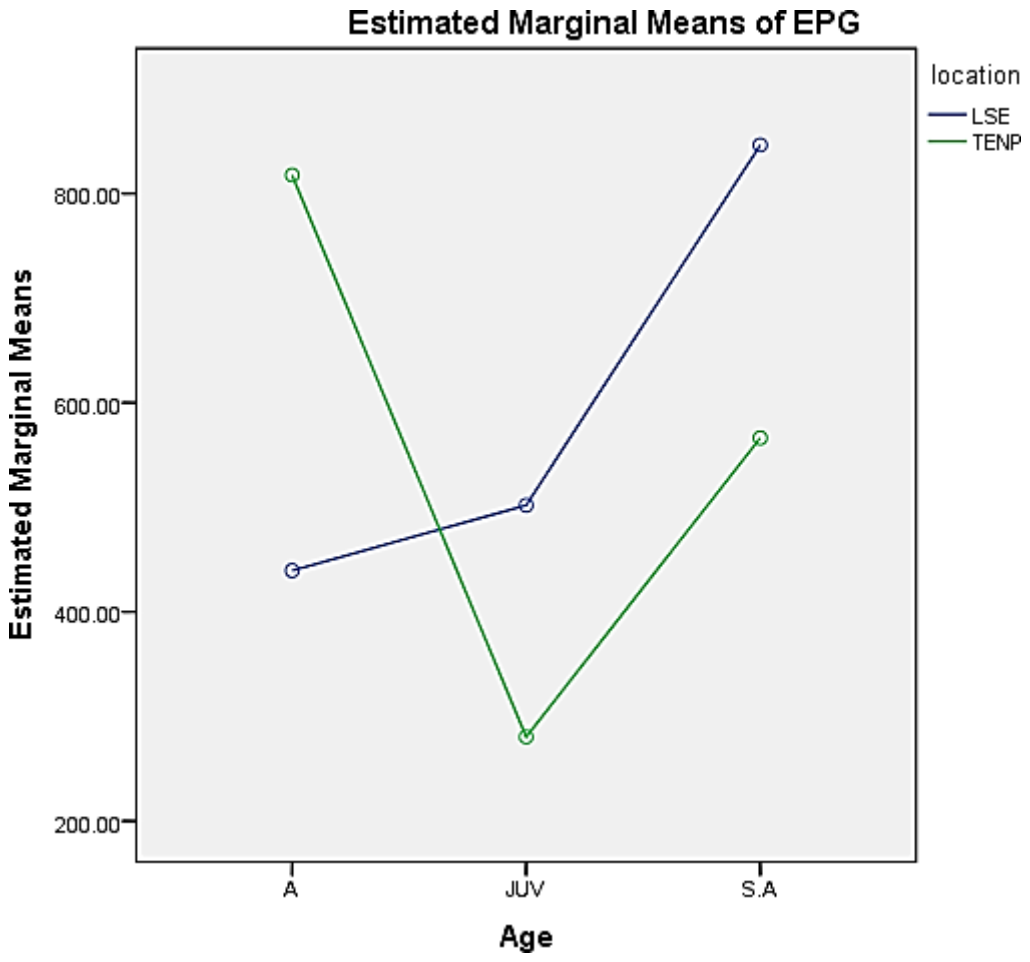


Figure 9: Plot showing the relationship between age and location against worm burden (EPG) in elephant populations from TENP AND LSE

According to this plot, based on location alone, though the difference between them was not statistically significant, adult elephants in TENP exhibited highest EPG means as indicated in Table 4, followed by sub-adults and Juveniles recording the least mean EPG. In LSE, on the other hand, adult elephants recorded the least mean EPG, followed by juveniles and sub-adults recording the highest mean EPG as shown in Table 4. The plot reveals that adults in TENP are more likely to experience higher intensity of worm burdens as compared to adult elephants in LSE. However Juvenile and sub-adult elephants in LSE are more likely to harbor more helminths than those in TENP.

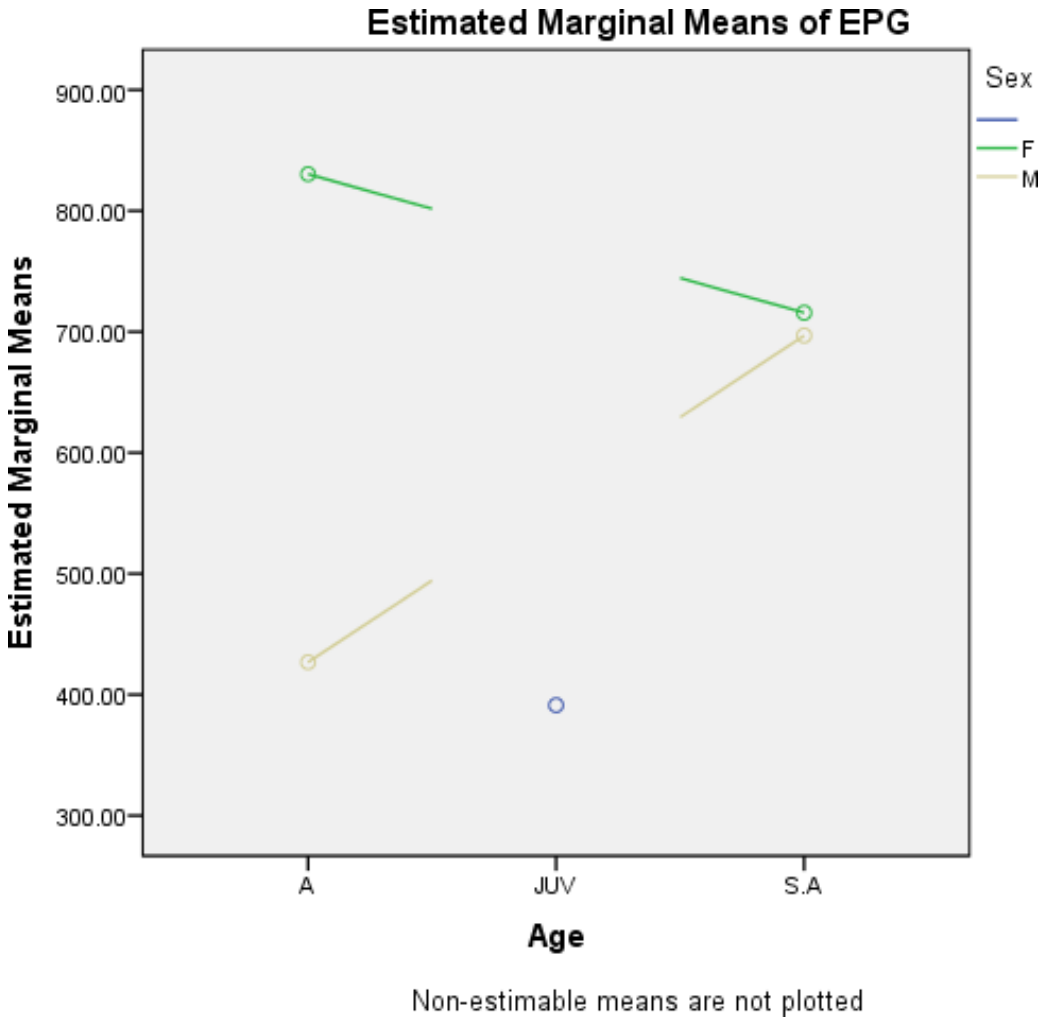


Figure 10: Plot showing relationship between age and sex against worm burden (EPG) in elephant populations in TENP and LSE

Infection intensity in adult females was higher (p= 0.016, CI 95%) compared to adult males while sub-adult males and females seem to have similar levels of infection intensity. Mean EPG in adult females was significantly different than that of sub- adult females same as adult males when compared to sub-adult males. It proved difficult to sex elephant calves while collecting samples in the field, hence the missing comparison of sex and age with regard to this group.

Table 5: Mean helminth burden (epg faeces) for each type of age group, social group and sex in African elephants from TENP and LSE populations in Kenya

Elephant population	N	Mean EPG ± SD	Median EPG
TENP	**137**	**623.06 ± 653.798**	**400**
Family social group	72	630.68 ± 591.977	425
Male social group	65	624.59 ± 741.542	400
Adult	87	733.73 ± 737.709	500
Sub-adult	30	566.67 ± 545.567	375
Juvenile	20	280.45 ± 247.252	225
LSE	**136**	**589.34 ± 589.237**	**400**
Family social group	106	685.85 ± 623.727	525
Male social group	30	248.33 ± 230.996	150
Adult	73	469.29 ± 536.717	300
Sub-adult	38	847.56 ± 648.648	750
Juvenile	25	502.00 ± 509.591	300
Total female EPG	**101**	**706.89 ± 625.963**	**600**
Total male EPG	**127**	**555.12 ±650.788**	**350**
TOTAL POPULATIONS EPG	**273**	**606.26 ± 621.558**	**400**

CHAPTER FIVE: DISCUSSION AND CONCLUSSION

5.1 Discussion

This study revealed that African elephants exhibit high prevalence rates of helminth infections and the patterns of infections observed vary within populations of TENP and LSE. The loss of and fragmentation of elephant habitats has led to splitting of populations to sub-populations due to restrictions in movement. King'ori *et al* (2020), in his study observed that these elephant sub-populations are likely to suffer different rates of parasite infestation. This study recorded prevalence rates of TENP (95.6%) and LSE (98.5%). This high prevalence rates correspond with what has been observed in other studies; (87.5%) by Elsheikha *et al.,* (2010), 97.5% reported by King'ori *et al*., (2020) in Kenya. This is an indication that African elephants are highly susceptible to helminth, especially nematode infections regardless of their age, social group or location. The overall nematode prevalence recorded was relatively high compared to trematode prevalence. A similar pattern between nematode and trematode prevalence was also recorded by King'ori *et al.,* (2020) with 97.5% and 39.1% respectively. Similarly, Baines *et al.,* (2015) recorded 73% nematode prevalence versus 26% trematode in a study in Okavango-Delta. The lower rate of trematode prevalence can be attributed to their complex life cycle.

Unlike nematodes, trematodes require an intermediate host (aquatic snails), whose presence is largely determined by presence of a permanent water source. This means therefore trematodes take longer to develop their infective stages and require special conditions, compared to nematodes, which undergo a more direct lifecycle (Elsheikha *et al*., 2010). Water resources in wild elephant habitats consist of permament and seasonal rivers, marsh/ swampy areas, and seasonal watering holes that dry up at times. This can attribute for the lower trematode prevalence as water is a requirement in the cycle of trematode infections.

Apart from the differences in the life-cycles of nematodes and trematodes, another factor that affects trematode prevalence is the abundance and distribution of aquatic snails within the ecosystem. Some of the factors that affect aquatic snail distribution include; physico-chemical water quality parameters like water temperature, dissolved oxygen, ions and salts in the water, depth, availability of food, predators, among others, all of which affect snail species distribution, in turn affecting trematode prevalence (Olkeba *et al*., 2020). In this study, elephants from TENP

recorded higher trematode prevalence than those from LSE (Table 2). This is unlike what was observed by King'ori *et al.,* (2020), where elephants from LSE recorded higher trematode prevalence than those from TENP. A malacological survey of wildlife habitats in Kenya, would therefore prove to be very useful in shedding more light on the distribution of trematodes in wild animal populations as trematode prevalence does not seem to be influenced by social group, but rather by location.

The presence of *Protofasciola robusta* and *Fasciola hepatica* can be attributed to the presence of marsh, swamps, streams and even possible watering holes that provide suitable environments for the intermediate snail hosts. If we consider that elephants feed in marshy areas, then possibilities of ingesting metacercariae are high. *P robusta* has been isolated from the duodenum and distal entrance of the bile ducts and small intestines of elephants (Condy, 1973 and Obanda *et al.*, 2011). The parasite has been associated with hemorrhage, intestinal tissue damage and calf fatality as observed by Obanda *et al.,* (2011). *Fasciola hepatica* adults are also found occupying the elephant's bile ducts and can lead to anorexia, constipation, jaundice, anemia and ultimately death. Fowler and Mikota, (2006) further explain that chronic infection could lead to obstruction of bile ducts, elevation of intrahepatic blood pressure, hypoproteinemia, hemorrhage and death. Therefore, scarttered water resources in the wild could explain the lower rates of trematode infections as elephants do enjoy lengthy periods of time in water to cool their body temperatures on hot days. Such behavior would the encourage higher rates of trematode prevalence, if water availability was not limited.

Elephants' grazing habits and extensive ranges they occupy encourage high rates of re-infection, accounting for the high nematode prevalence observed. During feeding, elephants were observed defecating in the same areas where they fed. These areas were also contaminated with dried dung, from elephants and other wild animal species that are found in these areas. In the dry season, it was observed that most adult elephants preferred to feed on shrubs and trees, due to unavailability of grass. Where grass was found, it was scanty, dry and very close to the ground. Elephants would use their trunk to collect grass, and as they did this, they would use their feet to try and remove dirt and soil from the grass before ingesting. Such feeding habits in an area that was contaminated with dung will most definitely lead to the ingestion of helminth ova, creating conducive conditions for infection and re- infection. This accounts for the high nematode prevalence rate recorded in

elephant populations.

The eggs of elephant hook worm, *Grammocephalus clathratus* were also isolated from the dung samples collected. Adults of this worm are found in the liver and bile ducts of the host, where they cause hemorrhaging and lesions (Obanda *et al.,* 2011). Heavy infestation in the bile ducts by these three species can lead to obstruction and eventually death. *Murshidia* and *Quilonia* species are found occupying the large intestines and on rare occasions, they can also be found in the small intestines (Condy, 1973). The pathology of these species has not been well described. It is important to remember that despite presence of intestinal parasites, healthy elephant populations are asymptomatic to helminth infections. However, in cases of starvation, helminths deprive host of nutrients, and in the course of their feeding, cause pathological lesions on the intestinal mucosa (Obanda *et al.,* 2011). This situation worsens in the case of heavy helminth infestations greatly compromising the elephants' survival fitness.

Obtaining adult worms from a host is either opportunistic (adult worms excreted during defecation), through invasive procedures or post mortem recovery. This could be obtained from rectal samples, when the animal has been immobilized for other medical interventions. This does not happen frequently as it is an invasive method, and when it does, often you may find the researcher absent and therefore no sample is obtained. The other alternative would be post-mortem recovery of adult helminths from the host. This opportunity is also very rare, for as explained by Baines *et al.,* (2015), elephants are accorded high level protection. Therefore, upon death, a post mortem is conducted almost immediately, to ensure quick and safe recovery of their tusks if present, and the carcass buried after.

In this study, opportunistic non-invasive methods were employed to obtain adult worms that were excreted together with dung. For these, morphology of anterior and posterior ends, total length of the worms, length and shape of oesophagus and shape of corpulatory bursa and morphology of spicules in males were used to identify genera present. It is important to note that helminth species, from same host are subject to extreme variations (Van der Westhuysen, 1938). This therefore creates uncertainties in classifying species. Following the method of Anderson *et al.,* (1974); Monnig, (1925) and Van Der Westhuysen, (1938), we classified the obtained samples up to the genus level to identify the genera *Murshidia* and *Quilonia.* These two species have been found

highly concentrated in the caecum and colon of elephant hosts by Condy, (1973). The possible pathological effects of these two species have been discussed earlier. Adult worms of the two genera have been recorded in elephants from Namibia by Thurber *et al.,* (2011), in Nigeria by Mbaya *et al*., (2013), and in Kenya by McLean *et al.,* (2012). Their eggs have also been identified by King'ori *et al.,* (2020) from Kenyan elephants across various populations. All these therefore makes the two genera the most commonly occurring genera of nematodes that infect African elephants.

Family social groups recorded higher mean EPGs as compared to the male social group, indication that the social structure in elephant populations does have an effect on intensity of helminth infections. The same was observed by King'ori *et* al., (2020). Family groups tend to associate in larger herds as compared to bachelor herds and lone bulls. This association in large herds creates an environment of re-infection as they tend to feed for long in the same areas and they defecate in these areas as they feed as explained earlier.

Another factor to consider when comparing infection intensity in the social groups is the foraging dynamics found in elephant social groups. Family herds rarely move far from water sources in dry seasons. This is because family herds consist of calves and sub-adults who may not move as fast as adults and are at higher risks of predation and mortality due to exhaustion. Lone bulls and bachelor herds on the other are not held back and can therefore travel further distances, in search of water and even better food in times of drought, with less risk (Wato *et al.,* 2018). Thus in the dry season, when family herds are utilizing dwindled and diminishing resources, the adult bulls are able to acquire better feed and water by traveling further. This therefore means that family herds will undergo nutritive and hydric stress more, leading to increase in intensity of helminth infections due to lowered immunity and thus record higher EPGs as compared to their male counterparts as observed in this study. Mean EPGs recorded reveal similar patterns of infection intensity, whereby female elephants' hosts are more parasitized than male hosts as observed by (Parker *et al*., 2020). However, other studies that have looked at the effect of sex on helminth infections in elephants yielded opposite results. In a study done at Chad Basin National Park in Nigeria, male elephants were found to be slightly more infected than their female counterparts (Mbaya *et al.,* 2013). According to their study, the higher levels of infection observed in male elephants can be attributed to some of their behavioral characteristics. Mature bulls tend to associate loosely with family social

groups, roaming from one family group to another looking for a female in oestrus for mating. As further described by Mbaya *et al.*, (2013), bulls in musth wet their trunk on the ground where a female elephant has urinated and even possibly defecated, and takes the trunk to their mouth, where they have a special organ (Jacob's organ), that is able to pick out pheromones, that indicate a female in estrus. Such behavior encourages direct ingestion of helminth eggs and even infective larvae that can increase infection intensity in males more than females. Foraging behavior described above, can therefore be assumed to counter the negative effects of this behavior, maintaining mean EPG in males at a lower level as compared to the family herd.

Mean EPGs recorded in this study, revealed relatively moderate levels of infection intensity by helminth parasites. Female elephants recorded a mean EPG of 707, while male elephants recorded 556. This is in line with other studies where female elephants recorded higher EPGs than males, as observed by King'ori *et al.*, (2020), where mean egg burden in female groups was 172 and in males was 89, numbers which are considered to be low. Baines *et al*., (2015) recorded a mean worm burden of 1116 in female elephants and 529 in male elephants from Okavango Delta in Botswana. Higher numbers have been recorded in Rhodesia by Van Der Westhuysen, (1938), where elephants recorded mean EPG of 2072.

Patterns in helminth infection in this study revealed that overall, elephants from TENP have a slight difference in mean EPG (623.06 ± 653.798) compared to elephants from LSE (589.34 ± 589.237) but this difference proved to be statistically insignificant. Using the General Linear Model (GLM), we were able to establish that adults in TENP had significantly higher EPGs as compared to adults in LSE. This may be explained by habitat range and resource distribution in LSE and TENP. TENP is a gazetted and fenced national park, covering approximately 12,000 km^2. The park has one main source of permanent water, Galana River, with seasonal sources including rivers Tiva and Voi, Aruba dam, scattered ponds, swamps and watering holes (Muteti *et al*., 2011). Muteti *et al*., (2011) noted that in the dry seasons, elephant home ranges in TENP shrink considerably as water resources become scarce. The elephants retreat to areas along Galana, Voi and Tiva rivers, to increase their chances of survival in the dry season. The reduction in home range therefore increases chances of heavy parasite infestation due to foraging in the same grasslands over a prolonged period of time. LSE, on the other hand, covers a much larger area of 33,817km^2 according to Ihwagi *et al*., (2015). The ecosystem has a wide range of habitats, associated with

climatic gradients within the region: hot and dry lowlands in the north, cool wet highlands to the south, interrupted with rugged mountains and open landscapes. The ecosystem allows for mostly free movement of elephants in between the different land uses due to the wildlife corridors maintained in these areas (Ihwagi *et al*., 2015). Elephant populations in LSE, therefore have access to a wider range of habitat as compared to those in TENP. During the dry season, elephants in LSE expand their home range in search for water and food, as was observed in Samburu National Reserve during the conducting of this study. Elephant data from Save The Elephants Foundation, included elephant families that were residents of the reserve, migratory herds, and new comers. This could be used to explain the differences in mean egg burden observed between the two locations. Elephants in TENP experience more stress in dry periods due to reduction of habitat range and water resources as compared to those in LSE, and therefore experience higher mean egg burden.

5.2 Conclusion

Helminth parasite families/species identified have been recorded to potentially cause harm to the elephant hosts in times of stress. As such, more studies are needed, to look into species variation within the elephants, up to the molecular level. This will increase our knowledge on parasite species diversity in the wild, which form a critical part of the elephant ecologic system. It would also provide more insight into the parasite-host equilibrium developed by helminths and the elephant host. High prevalence rates observed in both study areas should therefore be a point of concern when translocating at risk individuals, or when dealing with migratory herds, in order to minimize risk of infections and outbreaks. Patterns observed in this study revealed that apart from sex, the interaction of age and location has an effect on mean worm burdens experienced in elephant populations. More studies are needed to understand worm burden patterns in elephants within Kenyan ecosystems, in order to establish parameters such as ‘high worm burden’ and ‘low worm burden’. Such parameters are very important when conducting routine surveillance and health monitoring as one would have the data that other results may be compared to. This information would provide wildlife managers and veterinarians with information required, to guide medical interventions and other forms of interventions in the wild, to ensure optimum survival chances for

the African elephant species. If we look at the extent to which elephant habitats have been fragmented and lost, we see that some populations have been cut off from resources that they used to access. This therefore increases pressure on the few resources available that may not be enough to sustain the populations in times of drought. Taking the high helminth prevalence rates recorded in this study in healthy elephant populations, cases of disease and mortalities associated with helminth infections are bound to occur with adverse changes in climate, especially in calves. This data will also enable wildlife managers to identify populations that may be worst afflicted in times of adverse climate change, in order to monitor and intervene timely if need be.

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APPENDIX 1

Table 6: Mean, median and standard deviation of observed helminth counts obtained from the Sheather's sugar centrifugal floatation method.

		TENP	**LSE**
Total elephant population	N	137	136
	Mean ± SD	35.65 ± 44.88	28.5 ± 35.94
	Median	99	15
Familysocial group	N	72	106
	Mean ± SD	32.68 ±38.47	30.75 ± 38.35
	Median	18	15.5
Male social group	N	65	30
	Mean ± SD	38.91 ± 51.16	20.53 ± 24.57
	Median	19	14.5
Adult	N	87	73
	Mean ± SD	41.09 ± 49.21	28.75 ± 37.01
	Median	20	15
Sub-adult	N	30	38
	Mean ± SD	21.83 ± 32.28	28.45 ±37.4
	Median	9	16
Juvenile	N	20	25
	Mean ± SD	32.6 ± 37.67	27.84 ± 31.62
	Median	19	11

Table 7: Mean, median and standard deviation of observed helminth counts obtained from the modified water sedimentation method.

		TENP	**LSE**
Total elephant population	N	137	136
	Mean ± SD	27.45 ± 18.21	22.15 ± 21.43
	Median	22	15
Family social group	N	72	106
	Mean ± SD	22.78 ± 15.44	24.99 ± 22.69
	Median	20	17.5
Male social group	N	65	30
	Mean ± SD	32.63 ± 19.70	12.10 ± 11.81
	Median	29	8.5
Adult	N	87	73
	Mean ± SD	31.10 ± 18.32	19.33 ± 20.71
	Median	28	13
Sub-adult	N	30	38
	Mean ± SD	20.97 ± 18.18	30.82 ± 24.52
	Median	19.5	25.5
Juvenile	N	20	25
	Mean ± SD	21.30 ± 13.5	17.2 ± 14.2
	Median	20	14