PREVALENCE, RISK FACTORS AND GEOSPATIAL DISTRIBUTION OF *GIARDIA DUODENALIS* INFECTION IN DOGS IN NAIROBI COUNTY, KENYA

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF MASTERS OF SCIENCE IN APPLIED PARASITOLOGY DEGREE OF THE UNIVERSITY OF NAIROBI

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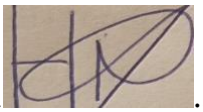
DECLARATION

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

Signed …  ..... **Date:** 15/4/2021

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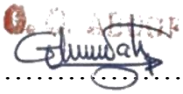
Signed……  …… **Date:** 19/4/2021

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DEDICATION

I dedicate this work to the almighty God for making it a success. To my loving husband Dr. Fredrick Musau, my daughters Monicah Ndunge and Olivia Muthoni, to my loving parents Mr. and Mrs. John Maingi and Mrs. Martha Musau and to all my brothers and sisters.

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LIST OF ABBREVIATIONS AND ACRONYMS

USA	United States of America
VSP	Variant Surface Protein
WHO	World Health Organization
DNA	Deoxyribonucleic Acid
PCR	Polymerase Chain Reaction
ELISA	Enzyme Linked Immunosorbent Assay
GPRS	General Pocket Radio Service
KSPCA	Kenya Society for Protection and Care of Animals
KVB	Kenya Veterinary Board
GPS	Global Positioning System
QGIS	Quantum Geographical Information System
UNICEF	United Nations International Children's Funds
HIV	Human Immunodeficiency Virus
AIDS	Acquired Immunodeficiency Syndrome
NIAID	National Institute of Allergy and Infectious Diseases

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ABSTRACT

Giardia duodenalis, the causative agent of mammalian giardiasis is a zoonotic parasitic infection that poses a serious public health risk. It is associated with diarrhea or loose stool in man and animals. The dog is as a host plays a crucial role in transmission of the infection since it enhances the maintenance of the cyst in the environment. It acts as a carrier, reservoir and transmitter of this parasitic zoonotic infection.

In Kenya, *G. duodenalis* has been detected and diagnosed in children although no animal assemblages were isolated. It has also been detected in cattle. However, there are no studies of the parasite infections in dogs and this study provided the first data supporting its prevalence, potential risk factors and the geospatial distribution in dogs in Nairobi County, Kenya.

This was a cross sectional study where faecal samples were collected from randomly selected dogs. A standardized questionnaire was administered to acquire data on risk factors. A multistage random sampling technique was used in the study. The veterinary clinics and KSPCA were the primary sampling units while systematic randomly selected dogs were secondary sampling units. Veterinary clinics sampled client-owned dogs while KSPCA was the catchment area for sheltered dog's population and the free roaming dogs.

A total of 400 faecal samples were collected from November 2018 to October 2019. The samples were then subjected to a *G. duodenalis* specific serological test called Giardia INDEX SNAP test to determine faecal antigen and hence its prevalence. An overall true point prevalence of 95% confidence interval for *G. duodenalis* infection was calculated using descriptive statistics.

Important risk factors for *G. duodenalis* infection for each individual dog that included age, ownership (owned vs. sheltered), breed, sex, neuter status, body condition score, faecal consistency, clinical signs and duration, type of food and method of feeding, area of origin,

season, nature of housing, purpose of keeping dog, vaccination status, co-infection and deworming status were captured using a standardized questionnaire. General Pocket Radio Service co-ordinates for each dogs' origin were generated. The heat map using the *Giardia* status illustrated the geo-spatial distribution of the disease in Nairobi County.

Univariate analysis for all the factors was done to determine their association to *G. duodenalis* infection. Factors with p value ≤ 0.2 were considered to have a significant association and were carried forward to the multivariable logistic regression model at a p value ≤ 0.05 while assessing for confounding and interaction.

The overall apparent prevalence of *G. duodenalis* in Nairobi County, Kenya was 22.25%. The univariate logistic regression analysis showed that 15 factors had positive univariable association ($P \leq 0.2$) to the occurrence and transmission of G. *duodenalis* infection. These were area of dog origin, ownership, breed, age, sex, vaccination and deworming status, body condition score, faecal consistency score, clinical signs, season, purpose of keeping the dog, type of food, dog husbandry and nature of housing. Neuter status and co-infections ($p > 0.2$) had no association with *G. duodenalis* infection and transmission.

In the final multivariate analysis model, only three factors namely; age, season and nature of housing that had a statistical significant association with *G. duodenalis* infection ($p< 0.05$) were retained. Young dogs (less than 12 months) had 0.22 (95% CI: 0.18, 0.40) times higher risk of getting *G. duodenalis* than adults (more than 12 months).

In the wet season, the risk of infection was 0.99 (95% CL: 0.39, 2.56) times more than in dry season. The probability of positive test result in dogs housed in kennel throughout and in dogs never housed being 1.61(95% CL: 0.82, 3.18) and 3.04(95% CL: 1.64, 5.65) times respectively.

There was a significant positive interaction between the age of the dog and the season of year to the occurrence of *G. duodenalis* infection. The risk of infection was high in young dogs in both wet and dry season while in adult dogs the risk of infection was only high in wet season.

The *G. duodenalis* infection was distributed throughout the County, but highly clustered in low-income areas such as Kawangware, Kibera, Mathare, Dandora, Kangemi, Kayole, Majengo and Ruai. Only 2 clusters namely; Kibera slum and Ruai sewerage collection point showed statically significant high risk of infection.

In conclusion, the study demonstrated that the prevalence of *G. duodenalis* in Nairobi County, Kenya was higher than what has been recorded in Africa and the overall prevalence worldwide. Age, season and nature of housing were the statistically significant factors associated with *Giardia* infection. The infection was distributed throughout Nairobi County but the risk of infection was higher in low income areas. The study recommends separation of young dogs from the adults. The Government and other stakeholder should also ensure improved sanitation through proper disposal of garbage and waste and to provide constant supply of clean water. There is need to educate dog owners on the importance of sheltering their dogs in order to control their movement and hence reduce contamination of cyst in the environment. The shelter facility should also follow the guidelines given by society of veterinarians to ensure health of animals by isolating new and sick animal in the facility, proper cleaning disinfection and removal of faecal waste and to routinely deworm the newly adopted dogs and others in the facilities.

CHAPTER ONE: INTRODUCTION

1.1 Background information

Giardia duodenalis, the causative agent of giardiasis is the most studied zoonotic parasitic infection that poses a serious public health risk to human. The infection has a significant public and veterinary health effect because of the high prevalence of infection and its ability to cause outbreaks. The infection present with clinical signs ranging from asymptomatic, acute to chronic disease. It is also the most commonly detected pathogen that is associated with diarrhea or loose stool in man and animals more so the domestic dog (Adell Aledón *et al.,* 2018). The infected dogs that are immune-competent act as carriers without showing clinical signs. Dogs that develop clinical signs of acute, chronic, intermittent or self-limiting diarrhea often lead to dehydration (Raza *et al.,* 2018).

The infection has a global distribution infecting both dogs and human (Ferreira *et al.,* 2013). In 2006, this protozoan parasite was included in the neglected disease initiative by WHO for being an important cause of diarrhea in children. This has increased the understanding of the parasite, its epidemiology and the disease burden globally (Puebla *et al.,* 2015).

Giardia has a direct life cycle. Once ingested, it replicate quickly in the small intestine through multiplication that result to cyst that are shed in the faeces of the host to the environment. The immense number of cysts voided per gram of faeces leads to high a risk potential to the risk of infection (Horton *et al*., 2019). A minimum of 10 cysts in faeces is enough to cause infection and infected individual can excrete billion of infectious cyst in faeces for some months. The transmission of the infection can occur directly by oral faecal route through ingestion of infective cyst in faeces of an infected dog or man or indirectly through ingestion of cystic contaminated food or water (Nguyen *et al*., 2018).

Cysts are hard due to the robust cell wall, are environmentally stable and are resistant to common form of water disinfectant such as chlorine. This makes them remain viable in the

environment and therefore cyst contaminated water become the main source of infection to both humans and dogs. However, the dog is the animal host that plays a crucial role in transmission of the infection because it enhances the maintenance of the cyst in the environment (Fantinatti, 2019). This is because dogs act as carrier and reservoir of this parasitic zoonotic infection (Gracenea *et al*., 2009). In addition, dogs are associated with caprophagic behavior. As a result, they ingestion cysts in faeces and therefore greatly contribute to the transmission of *G. duodenalis*. This result to infection also referred as giardiasis and/or to eventual clinical symptom i.e. giardiosis (Fantinati, 2019).

Unsheltered dogs move out and about wandering around favoring the dispersion of the infectious cyst in the environment. Infected pet on the other hand contribute to the transmission within the homestead infecting man and other domestic animals. The risk of infection is high in crowded places such kennels, dog in shelter facilities and animal orphanages. This is because, large number of dogs in kennel facilities and shelters lead to overcrowding which result to stressful environment and therefore increasing the risks of infection (Fantinatti, 2019).

The infection has a worldwide distribution causing giardiasis in both animals and humans in developed and developing countries. In developed countries the prevalence of infection ranges between 2 - 7% while in developing countries its between 20 - 30% (Ferreira *et al.*, 2013). The high prevalence in developing countries is because of poor sanitary conditions that contaminate water and food with cyst (Puebla *et al*., 2015). A high prevalence of up to 100% has been reported in kenneled dogs (Pipia *et al.*, 2014).

There are several common factors associated and predispose dogs to infection. These include the age, sex, co-infection, stress, immune status, nutrition, animal density per kennel, breed, season, living conditions, gut microbiome (Mircean *et al*., 2012; Uitawijk *et al*., 2019). Young and immuno-compromised dogs have a higher risk of infection as compared to adult

and immune competent dogs. They also exhibit pronounced clinical signs which are observed within 2 weeks after ingestion of infectious cysts (Mravcova *et al*., 2019). The type of gut bacteria present may also enhances the vulnerability of the infection to the host (Horton *et al.*, 2019).

World Health Organization estimates that, about 3.5 billion people live in areas with poor sanitation. From this population, 1 billion people globally are infected with *Giardia* (WHO and UNICEF, 2008). About 2.5 million of these individuals come from the developing country (Laishram *et al*., 2012). The infection is high in areas with poor sanitation, lacking constant supply of clean water and proper sewerage disposal. This high prevalence was linked to poverty in undeveloped countries and the lack of knowledge of molecular mechanisms for the disease (Savioli *et al*., 2006). Therefore, cyst from faecal waste of an infected person can contaminate water bodies and remain infectious for a long period (Leclerc *et al*., 2002).

In humans, the parasite has been described as a frequent pathogen occurring annually with up to 280000 deaths estimated cases worldwide. This high mortality rate has made *Giardia* to be classified as NIAID Category B Priority Pathogen hence added as a neglected disease by World Health Organization.

In Kenya, G. *duodenalis* has been documented in livestock in Kisumu County at a prevalence of 14% (Kanyari *et al*., 2010). It was also diagnosed in children faeces by molecular characterization, but animal related assemblages were not detected (Mbae *et al*., 2016). There are no dog related studies and therefore the data supporting the parasites prevalence, potential risk factors and the geospatial distribution are scanty. This study therefore, provided for the first time the prevalence, risk factors and geo-spatial distribution of *G. duodenalis* infection in Nairobi County, Kenya.

1.2 Problem statement

Giardia duodenalis is of serious public health importance worldwide and has been recorded in livestock (Kanyari *et al*., 2010) and humans (Mbae *et al*., 2016) in Kenya. Despite its serious health risk, the disease has however remained neglected. In order to control and prevent canine and human giardiasis, it is important to detect and characterize the *G. duodenalis* parasite in faeces. Many studies elsewhere have detected the parasite in dogs by ELISA test and molecular tests, therefore providing information that has led to the understanding of the epidemiology of infection in others region of the world. However, this information is missing in Kenya and therefore the epidemiology of the disease in dogs is unknown. This study therefore detected the *G. duodenalis* in faeces of dogs and provided the data on prevalence, risk factors and the geo-spatial distribution of the infection in Nairobi County.

1.3 Justification

Giardia duodenalis is a zoonotic parasite, a major cause of water and food borne diarrhea disease and of major public health importance. In Nairobi County, there is an increased human population with an estimated population density of over 3,017 persons per square kilometre. This has led to development of slums, poor sanitary condition, inadequate constant supply of water and poor sewerage disposal. At the same time, the majority of these people living here have adopted and kept dogs as pet and for security purposes. These practices have led to increased dog population but unfortunately, most dogs are not well managed while others become free roaming. Some of these dogs have ended up in shelter facilities.

Despite dog acting as natural transmitter of this pathogen, there is no data on the occurrence, identity and prevalence in dog in Nairobi County, Kenya. Therefore, the zoonotic potential of this protozoan parasite cannot be underestimated. In addition, the diagnosis of this infection has been missing in veterinary medicine as a routine practice for the basis of the

treatment. This study focused on the investigation of *G. duodenalis* infection and provided current data for the first time on prevalence, risk factors and the geospatial distribution of *G. duodenalis* in dogs in Nairobi County Kenya.

This data will be fundamental in sensitization and creation of awareness to veterinarians, dog owners, and relevant authorities in veterinary and public health departments and as well help in future control measures in Nairobi Kenya. The data will also help in public health as giardiasis will also be included as a differential in managing diarrhea diseases in children especially from low income areas.

1.4 Objectives

1.4.1 General objectives

To determine, the prevalence, risk factors and the geo-spatial distribution of *G. duodenalis* infection in dogs in Nairobi County, Kenya.

1.4.2 Specific objective

i. To determine the prevalence of *G. duodenalis* infection in dogs in Nairobi County, Kenya
ii. To identify potential risk factors associated with *G. duodenalis* infection in dogs
iii. To determine the geospatial distribution of *G. duodenalis* infection in dogs

CHAPTER TWO: LITERATURE REVIEW

2.1 *Giardia*

2.1.1 Taxonomic classification

Giardia belongs to the phylum Sarcomastigophora, class Zoomastigophora and order Diplimonadida. It is a single celled eukaryotic and extracellular protozoan parasite of the upper intestinal tract of humans and animals worldwide. Its taxonomic classification is complex and confusing. Recently, it has been classified by molecular methods based on the host and the morphomolecular characteristics. The first subdivisions of assemblages were based on host specificity by intrinsic characteristics such as antigenic characteristic, isoenzyme and DNA analysis (Feng and Xiao, 2011; Fantinatti, 2019). The parasite was then clustered into 7 distinct genotypic groups called assemblages (A – H) which infect specific hosts. Assemblages A/B affects humans, C/D affect dogs, E affect hoofed animals, F affect cats, G affects rats and H that affect sea animals (Fantinatti, 2019).

Based on these assemblages, there has been proposals to have a logical sequence in the taxonomy in order to reduce confusion and therefore, to adopt *G. duodenalis* for assemblage A, *G. enteric* for assemblage B, *G. canis* for assemblages C and D, *G. bovis* for assemblages E, *G. cati* for assemblages F and *G. simondi* for assemblage G (Feng and Xiao, 2011).

Other recognize the 7 assemblage as distinct species of *Giardia* although the literature about most of them is scanty (Fantinatti, 2019). However, 6 separate species have been documented to exist with 5 known to have host specificity. These are *G. agilis* found in amphibians, *G. microti* in moles and muskrats, *G. ardeae* and *G. psitacci* infecting birds, *G. muris* in rodents and *G. duodenalis* also *G. lamblia/ G. intestinalis* that infect a wide range of mammalian host including wildlife, livestock, companion animals and humans. *Giardia duodenalis* and *G. muris* used in experiment with laboratory animals are the most studied species (Mark- Carew *et al.,* 2013; Fantinatti, 2019).

2.1.2 Cellular morphology

Giardia is an extracellular protozoan parasite that has characteristics that resemble anaerobic prokaryotes. Therefore, it lacks common eukaryotic subcellular compartments such as peroxisomes mitochondria and Golgi apparatus (Mark-Carew *et al*., 2013). The protozoa exists in two morphological life stages i.e. trophozoite and cyst.

The trophozoite main replicative stage, is motile and pear-shaped, measures 10–20 μm by 5–15 μm, and has two functionally identical and transcriptionally active diploid nuclei anteriorly, median body, and four pairs of flagella i.e. anterior, posterior, caudal and dorsal. On its ventral side lies a concave sucking disc comprised of ultra-structurally repeating units of microtubules for attachment to the intestinal wall (Laishram, 2012). They are entirely covered by variant surface protein (VSP) expressed one at a time to the host immune system enabling it to infect various host species (Ropolo *et al*., 2005).

In adverse conditions, the trophozoite releases cyst wall protein on its surface. This are packaged in encystation vesicle and is incorporated in the outer protective covering inside which the nucleus replicates to form 4 nuclei (Chavez *et al*., 2007). It is oval shaped, measures 11–14 μm by 7–10 μm, has a thick outer shell, a central axostyle and four nuclei (Laishram 2012). It has two mitotically arrested trophozoites with a thick tough double layer hence environmentally resistant and serves as the infective form (Chavez *et al*., 2007).

2.1.3 Life cycle

Giardia has a direct lifecycle. After ingestion of the infective cyst, the low pH, gastric acids and pancreatic hydrolytic enzymes cause the cyst to undergo excystation at the duodenum resulting into formation of an excyzoite that divide into four trophozoites (Mark Carew *et al*., 2013).

The trophozoites use sucking disc to attach on basal aspect of the brush border of the proximal small intestine and absorb nutrients through the cell membrane. They then multiply

by longitudinal binary fusion producing large numbers seen in a classical infection. Some trophozoites undergo encystations in the ileum or as they move towards the colon and form cyst, which are then shed via faeces. However, in cases of heavy infestation and gut hypermotility, some trophozoites can be expelled before encystations but they are not infective once released because they decompose very fast in the environment (Laishram *et al*., 2012).

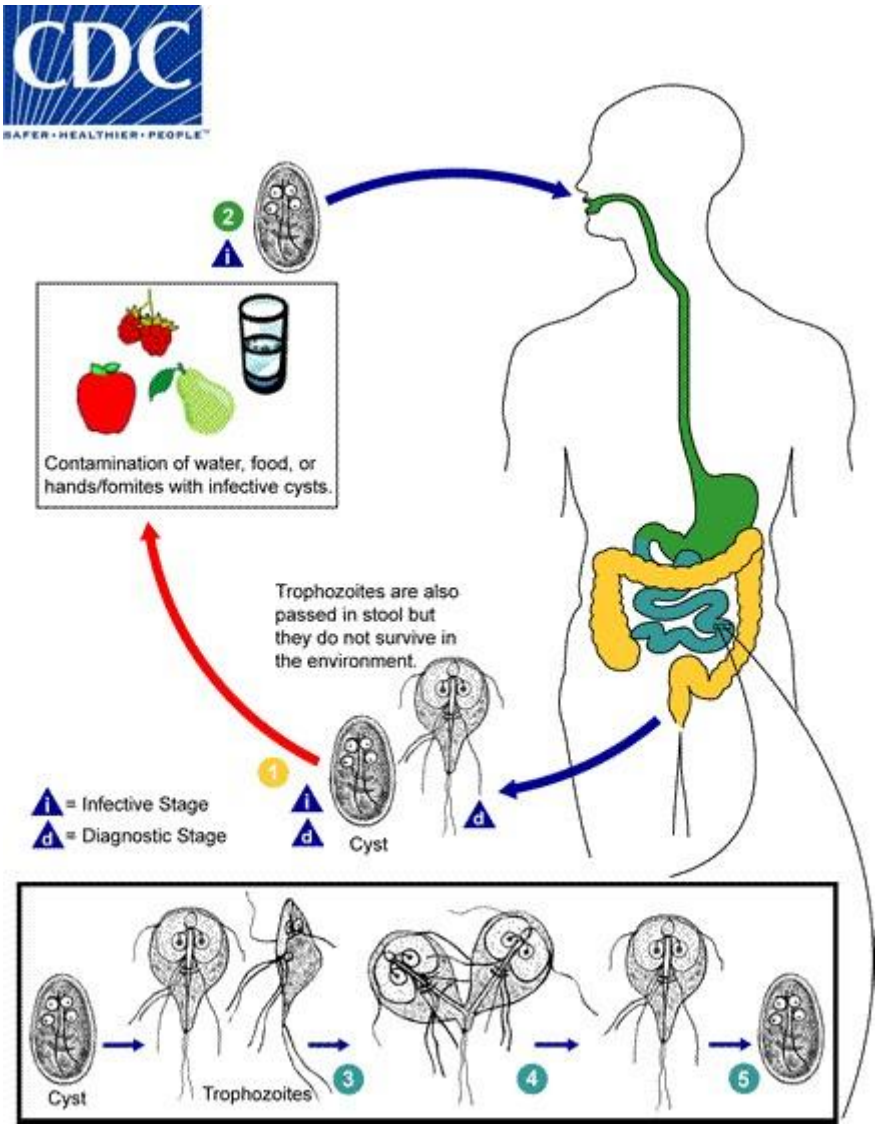

Figure 2.1: The life cycle of *Giardia duodenalis* in man (Center for Disease control and prevention, 2012)

2.2 Giardiosis

2.2.1 Aetiology

Giardiosis is caused by *G. duodenalis* also known as *G. lamblia* or *G. intestinalis*. This is an enteric protozoan parasite that is omnipresent, causing an emerging protozoa disease of public health importance. The disease is a leading cause of diarrhea in children and dogs. It is associated with co-morbidities, which affect their health and welfare considerably (Chavez *et al*., 2007).

2.2.2 Transmission

Transmission is usually through ingestion of water and or food/ feed contaminated by *Giardia* cysts. Cysts are infectious when shed in faeces or shortly after. The transmission can occur immediately if ingested by a new host or by the same host, with as low as 10 cysts resulting in patent infection (Adam *et al.,* 2016). Dogs can also be re-infected while grooming their body fur (Centre for food security and public health, 2012). Once infected, an animal or human can shed billions of cysts in their faeces. The prepatent period for giardiasis is between 5 - 10 days for dogs and up to 16 days in cats (Bowman *et al*., 2002), while in human loose stool appear 7 - 10days after infection. The cysts are extremely hardy and can survive for long periods in the water **(**Mossalanezhad *et al.,* 2010).

2.2.3 Virulence and pathogenicity

Giardia is associated with maldigestion and malabsorption that develop subsequent to epithelial cell apoptosis, barrier and transport dysfunction, inhibition of lipases and disaccharidases, and physical disruption of the microvillar glycocalyx (Laishram *et al*., 2012). This results to increased epithelial permeability from disruption of peripheral membrane proteins. These are the tight junctions-associated protein zonula occuludins-1 (Mark-Carew *et al*., 2013). This triggers host inflammatory response that causes; brush border injury, disaccharidase deficiencies and blunting of intestinal villi and microvilli

resulting in decreased surface area for absorption of nutrients, impaired active transport and accelerated exfoliation hence fluid accumulation in the intestine and diarrhea. Infection can also result to post *Giardia* chronic intestinal disturbances such as irritable bowel syndrome and inflammatory bowel syndrome (Laishram *et al*., 2012). Additionally, *Giardia* is also known to trigger enterocyte apoptosis (Mark-Carew *et al.,* 2013).

There is of also decreased enzymatic activity in the host that results in absorption of zinc, which is an irreplaceable element in immunological reactions. This is because *Giardia* infection leads to diarrhea and damage to the intestines which can also lead to zinc excretion. Therefore, zinc deficiency that has an immunosuppressive effect result in increased susceptibility to infections. In addition, availability of zinc modulates the immune system and if its availability is altered there is increased susceptibility to gastrointestinal protozoan pathogens. *Giardia* parasite has also been found to compete with host for the availability of zinc. This is because the surface and the flagella are covered by surface proteins which are capable of binding metal such as zinc. These VSP inhibit the functions of zinc once it's bound (Mravcova *et al*., 2019).

2.2.4 Clinical signs in dogs

The incubation period ranges between 5-12 days and the clinical signs start to appear after 14days following the ingestion of the infective cyst (Mossalanezhad *et al.,* 2010). Infections depend on parasite and host factors. Parasite factors include number of cyst ingested, ability of the parasite to adhere, strain and virulence of the parasite while host factors are PH of gastric juice, immune status, intestinal microbiome and nutrition (Trevisan *et al.,* 2020). The infection is usually subclinical runs a latent course.

Acute giardiasis can occur after an incubation period of 1 -14 days and can lasts from one to three weeks (Mark- Carew *et al*., 2013). The dog may develop nonspecific signs including severe enteritis, malodorous diarrhea, steatorrhea and weight loss or failure to gain weight

(Laishram *et al*., 2012). There may be alternating periods of diarrhea and constipation (Mravcova *et al*., 2019). The diarrhea can be self-limiting in immuno-competent dogs (Centre for food security and public health 2012). Occasionally, vomiting occurs, but fever is not usual. In chronic infections, diarrhea can appear with or without an acute phase usually due to recurrence from failed treatment. This chronic disease can last for long more so in immune compromised dogs (Bianciardi *et al*., 2004).

2.3 Prevalence of *G. duodenalis*

In order to understand the disease burden, it is important to know the aspect of host range, species, strains and the potential for cross species transmission. According to WHO, its estimated that approximately 200 million humans' cases shows symptomatic giardiasis in Latin America, Asia and America while there is an estimate of 500000 new cases reported annually. In Cuba, giardiasis is an endemic parasitic infection with up to 55% prevalence rate (Puebla *et al*., 2015). In dogs, the prevalence varies by age, method used for diagnoses, ownership and the immune status of the animal. The prevalence is also high in dog housed in large numbers such as in kennels and shelters (Quadros *et al.,* 2016).

In Kenya, a prevalence of 14% has been reported in livestock in Kisumu County (Kanyari *et al* 2010) and 4.5 % in humans (Mbae *et al*., 2016). However, in dogs the prevalence is still unknown. In other parts of the world, prevalence of up to 15.6% has been reported in dogs in USA (Carlin *et al*., 2006), 8.6% in Vietnam (Nguyen *et al.,* 2018), 13% in Canada (Olson *et al.,* 2010), 14.3% in China (Qi *et al.,* 2016), 21% in central London (Upjohn *et al.,* 2010) and 25% in Trinadad and Tobego (Mark-Carew *et al.,* 2013). In Africa, canine giardiasis also shows variable prevalence rate, ranging from 1.7% in Egypt (Ahmed *et al*., 2014), 5.6% in South Africa (Mukaratirwa and Singh, 2010) to 17.4% in Nigeria (Abubakar *et al.,* 2015).

2.4 Risk factors for *G. duodenalis* transmission

2.4.1 Risk factors in dogs

Several factors are known to predispose dogs to *G. duodenalis* infection and they include age (Mark-Carew *et al*., 2013), breed (Upjohn *et al*., 2010); neuter status, season and area of origin (Mohamed *et al.*, 2013), ownership (Private owned verses store owned), gender (Nguyen *et al*., 2018) and purpose of keeping dog (Abubakar *et al*., 2015). Co-infection, stress, immune status, nutrition, animal density per kennel, season, living conditions and gut microbiota are also associated with *G. duodenalis* infection in dogs (Mircean *et al*., 2012; Uitawijk *et al*., 2019).

In Romania, the risk of infection was high in young dogs less than one year at 47.1% than in older dogs 28.3% (Micearn *et al.*, 2012). Elsewhere, risk of infection was high in stray dogs at 38% in Colombia (Pulido-Medelin *et al.*, 2019) and at 67% in Ireland (Horgan *et al.*, 2020). This is due to poor health control and the dogs are in continuous contact with other infected dogs excreta in the environment and are immunosuppressed. Kennelled dogs had a prevalence of 50% in Romania (Mircean *et al.*, 2012). Male dogs have also been reported to have a high risk of infection at 56% than females at 32% in Colombia (Pulido-Medelin *et al.*, 2019). A weak immune response as well as co-infection by other parasites and/or pathogenic bacteria increases morbidity and mortality. In temperate countries, the infection is also higher during winter season compared to summer (Palmer *et al*., 2008; Gracenea *et al.*, 2009).

2.4.2 Risk of zoonotic transmission

Giardia duodenalis assemblages though fairly host specific have a high zoonotic potential. Studies have shown that cysts from asymptomatic children when cultured in the laboratory and then the cysts and trophozoite fed to dog, they start shedding cysts in 5 - 6 days**.** It has also been observed that assemblage A and B known to infect humans also infect dogs and other domestic animals while other isolates affect more than one host hence zoonotic

potential (Lalle *et al.,* 2005). In nature, dogs are known to harbour both zoonotic and *Giardia* specific assemblage infections hence are natural reservoir for infection in humans (Quadros *et al*., 2016).

An infected animal can excrete up to 1 billion cysts in faeces every day for up to one month, which are infectious upon ingestion. The cysts are usually environmental stable and therefore can live in water, food/feed and surfaces for a long time (Adam *et al*., 2016). The risks of zoonotic transmission are high in developing countries due to high populations living in slums with poor sanitary condition, inadequate supply of clean water, poor sewerage disposal and increased number of roaming dogs resulting to cysts contaminating water and environment. Further, such setups harbour more people including children and patients suffering from malnutrition, immunosuppressive diseases like HIV and AIDS who are at high risk of *Giardia* infection (Zhang *et al.,* 2017).

2.5 Diagnosis

Giardiasis is diagnosed presumptively through observation of clinical signs manifested by the animal or by microscopic detection of the cyst. Other methods are serological and molecular methods.

2.5.1 Microscopic diagnosis

Previously, *Giardia* was diagnosed by use of microscopic identification of either cysts, trophozoites or both in faeces. This has been through direct faecal smears or fecal wet mounts are used when stained with methylene blue dye to help in visualization and identification of trophozoites and cystic structure or by direct floatation method that uses zinc sulphate solution or sugar solution in the floatation method but is optional in the detection of *Giardia* cysts. Zinc Sulphate has a specific gravity of 1.18 and preserves the cysts morphology unlike the sugar solution with a specific gravity of 1.27. The high specific gravity of sugar solution distorts the cysts making it more difficult to identify them during faecal floatation (Fantinatti,

2019). Cysts can also be concentrated by passive or centrifugal fecal floatation when trophozoites are not visible on direct smear (Centre for disease control and public health, 2012).

Although microscopic examination is the commonly method used in most hospitals laboratories and veterinary clinics, it has been found to be time consuming and need an experienced microscopist. It also has a low sensitivity of 48.2% but a high specificity of 99.5% (Uiterwijk *et al*., 2018). Trophozoites are relatively easy to identify under microscopy because they are motile in wet smears but are very fragile and decompose very fast in the environment. However, the identification of cysts is easy but challenging owing to their very small size and transparent nature (Carlin *et al.,* 2006).

Faecal samples may show a small number of the evolutionary *Giardia* forms, which can prevent or even mask the host load of parasite. Negative false cases can also occur, due to the intermittent elimination of the protozoan cysts (Fantinatti, 2019). These factors coupled by inexperience makes diagnosis of *Giardia* very challenging and could be contributing to under reporting of this disease in many parts of the world including Kenya.

2.5.2 Serological diagnosis

This involves use of more sensitive test for keen examination of faecal samples and accurate diagnosis of this disease in dogs and humans. The common serological tests are immunoenzymatic method or by immuno-chromatography, direct immunofluorescence assay and immuno-flurorescence antibody test (IFAT). These test have greater sensitivity, specificity and reproducible for single sample (Fantinatti, 2019). They are also easy to use, easily interpretable and cost effective (Mark -Carew *et al.,* 2013). However, they are expensive and time consuming and need to be analyzed by a specialized **(**Mossalanezhad *et al*., 2010).

The commonly used method is an immuno-chromatography assay. This rapid enzyme immunoassay antigen test kit that can be used on fresh faeces or previously frozen faeces. The test involves utilization of *Giardia* cyst wall antigen as a diagnostic test that has a sensitivity of 85 - 98% and specificity of 90-100% (Mossalanezhad *et al.,* 2010**)**. This test is an ELISA based test that contains an antibody-specific to *Giardia* cyst wall antigens released into the faeces during encystation by a substrate solution that binds with the antigen in the test chamber forming a conjugate. The conjugate then interacts with the substrate solution generating a blue colour that denotes a positive sample (Carlin *et al*., 2006).

2.5.3 Molecular diagnosis

The molecular diagnosis of *G. duodenalis* has developed due to their high sensitivity and specificity; molecular diagnostic tests have increased the identification of the various diversities. These involve the use of Polymerase Chain Reaction (PCR), which is more sensitive for detection of *G. duodenalis* genes (Fantinatti, 2019). It has an average sensitivity of 92% and a specificity of 100% (Kwannan, 2007; Mravcová *et al*., 2019). Researchers have shown that PCR is the most sensitive and specific test available and it allows for genetic analyses of the *Giardia* assemblages and sub-assemblages in a single stool sample (Mark-Carew *et al.,* 2013).

There are two methods of molecular diagnoses by PCR. These are the semi-nested PCR amplification protocol and the TaqMan real time protocol. A higher sensitivity is achieved by use of semi-nested PCR. Unfortunately, it is not used for routine diagnoses because there is always risk of contamination by short amplified DNA segments. However, this risk has been eliminated by the use of the TaqMan real time protocol (Adamska *et al.,* 2010).

The genotypic specificities is done on characterization of β-giardin, small subunit ribosomal RNA (SSU-rRNA), triose phosphate isomerise (*tpi*) genes and glutamate dehydrogenase (*gdh*). Glutamate dehydrogenase gene is more used in the analysis of the *G. duodenalis*

(Malekifard and Ahmadpour, 2018). Inhibitors in the faecal specimen and the difficulty of in cyst disruptions (Kwannan *et al*., 2007) have challenged the sensitivity of PCR diagnostic method.

These faecal inhibitors include; complexity of the faecal sample where the *Giardia* parasite is found, the genetic material to be isolated is mainly enclosed in the cysts, which has a robust cell walls. Lastly, heme, bilirubins, bile salts, and carbohydrates constituents in the faeces inhibit PCR by impairing cysts lysis, degrade the nucleic acid and inhibit activity of polymerase if co-extracted together with the target pathogen DNA. These factors have limited the use of method in human and animals because of the adoption of the prior faecal processing which add on labor, cost and time hence making the method more expensive. At the same time, concentration and the purification processes has been shown to result in the loss of cyst in the fecal material (Hawash, 2014).

2.6 Treatments

Currently, there is no drug labeled for the treatment of canine giardiasis. Treatment is less effective in dogs with hypermotile diarrhea as this increases gastrointestinal transient time, which minimizes drug-trophozoite interaction time (Carlin *et al*., 2006). However some drugs such as metronidazole have been use although it has been associated with some adverse side effects such as nausea, vomiting, diarrhea, in appetence and neurological dysfunctions (Moron-Soto *et al*., 2017). Albendazole has also been tried but it has been reported to suppress the bone marrow, cause bloody diarrhea, teratogenicity and abortion. Febantel which is a combination of fenbendazole, praziquantel and pyrantel is another drug that has been used against *Giardia* (Moron-Soto *et al*., 2017). Nitazoxamide is a recent drug which has been tried and approved by FDA against *Giardia*. The drug has been document to reduce cyst shedding in infected dogs when administered every 14 days at a dose of 75mg/kg for 3 consecutive days. (Moron-Soto *et al*., 2017).

2.7 Prevention and control

Giardia infection can be prevented by avoiding the ingestion of infective cyst in the environment. Faeces in the environment should be removed to avoid contamination with the cyst that will eventually lead to infection in other dogs and humans. *Giardia* cysts can be inactivated on surfaces through cleaning and disinfection with quaternary ammonium compounds and/or chlorohexidine (Tangtrongsup, 2013).

The control strategies should focus on hosts involved in the transmission of the giardiasis. This will help curb the circulation and transmission of the parasite and reduce the disease. However, the effective control should be prioritizing on the basic sanitation and education of people on maintaining public health for self-prevention of the disease (Fantinatti, 2019).

CHAPTER THREE: MATERIALS AND METHOD

3.1 Study design

This was a cross sectional study where faecal sample from systematically randomly selected dogs were collected directly from the rectum. They were then subjected to a *G. duodenalis* specific serological test in the laboratory to determine their *Giardia* status.

During sample collection, a standardized questionnaire was administered in order to acquire data pertaining to risk factors. General Pocket Radio Service (GPRS) co-ordinates of the respective homes where sampled dogs lives, were generated and were used together with *Giardia* positivity results, to generate heat map illustrating the geospatial distribution of the disease in Nairobi County.

3.2 Study area

The study was conducted in Nairobi County (**Fig 3.1)**; the capital city of Kenya. Nairobi County was purposely selected as it has; the highest population of dogs both owned and free roaming with an estimated population density of over 3,017 persons per square kilometre and about 50000 strays dog (Kenya news agency 2021). The County has an average annual rainfall of about 925 mm with ambient temperatures ranging from 12 – 24^{o}C. It also has the highest number of registered veterinary clinics currently at standing at 21 and the increasing anecdotal reports from hospitals and veterinary clinics on giardiasis outbreaks in dogs and humans' populations.

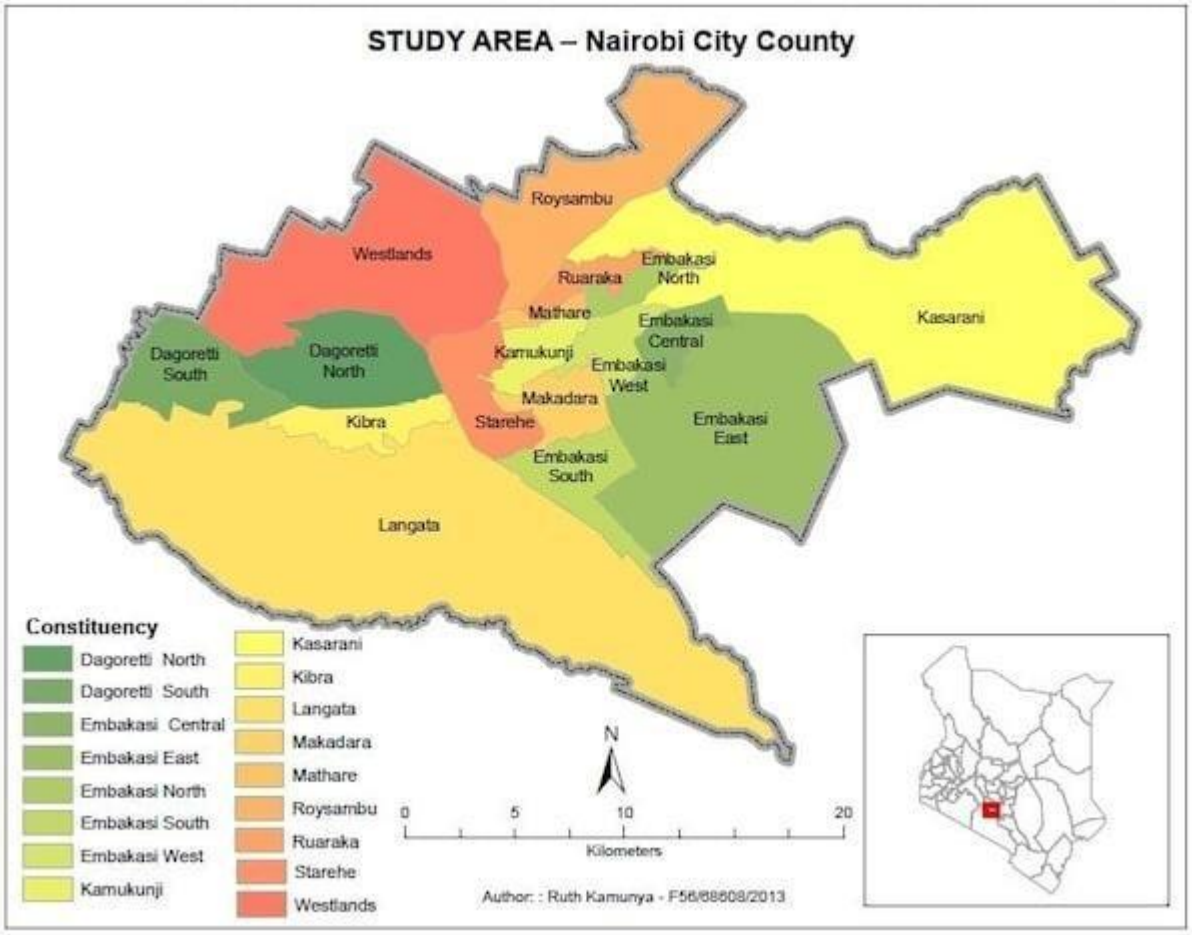

Figure 3.1: The map of Nairobi County, Kenya (www. researcghgate.net)

3.3 Sample size calculation

The sample size was calculated based on a prevalence of 17.4% reported in a similar study done in Zaria, Nigeria (Abubakar *et al.,* 2015) at 95% level of confidence and 5% error of estimation. This study was selected as Zaira had comparable epidemiological factors (economic, level of veterinary care, climate, human and dog population) to Nairobi County. The formula described by Dohoo *et al.,* (2003) as shown below was applied.

$$n = \frac{Z_{0.05^2} * pq}{L^2}$$

L= 0.05 margin of error,

p= 0.174 is the prevalence of canine giardiasis found in Nigeria,

q = 1-p = 0.826

$\mathbf{Z}_{0.05}$ is the normal deviate from the mean in Z distribution =1.96.

Based on this calculation, the minimum sample size required was at least 221 dogs. The number was then increased by 10 dogs for every risk factor evaluated. As there were 17 risk factors assessed in this study, an additional 170 dogs were included raising the sample size to 391 dogs. For convenience purposes, this figure was rounded off to 400 dogs (Dohoo *et al.,* 2003).

3.4 Selection of sampling unit

A multistage random sampling technique was used in this study. Veterinary clinics and the Kenya Society for Protection and Care of Animals (KSPCA) were the primary sampling units as they are sampling unit to be selected in the first stage. Systematically randomly selected dogs were the secondary sampling units. Veterinary clinics were used to sample client-owned dogs while KSPCA the shelter dog's population. Kenya Society for Protection and Care of Animals was purposely selected as it is the only shelter facility in Nairobi County that

provide shelter or temporary home to lost dogs, stray and surrendered dogs before they can be reclaimed, adopted or rehomed.

In order to identify veterinary clinics where client-owned dogs were to be sampled from, a list of all registered veterinary clinics in Nairobi was obtained from the Kenya Veterinary Board (KVB) database and used as the sampling frame. The clinics were first stratified into 4 regions (North, South, East and west) based on their respective location within Nairobi County. One veterinary clinic in each region was then selected through simple random selection. The selected clinics were Kiambu Road Vet. Clinic, Andy's Vet. Clinic, Jacaranda Vet. Clinic and the University of Nairobi Animal Hospital. Formal requests were then sent to the head veterinarian in the selected clinics inviting them to take part in the study. Trained research assistant were then sent to the selected veterinary clinics and KSPCA in order to assist in the sample collection.

The number of dogs to be sampled from each clinic was proportional to the number of dogs treated per year in the selected veterinary clinics and KSPCA. To identify this, participating practices were requested to provide an estimate of the number of dogs treated in the preceding year. The total number of dogs was then calculated, and the respective proportion of study animals computed using the formula shown below:

Number of dogs to be sampled per clinic **= {y÷ (k+n+s+w+e)} 400**

Where;

y = Number of dogs treated in a selected clinic/KSPCA per year (either be **k/n/s/w** or **e**)

k= Number of dogs treated in KSPCA per year.

n= Number of dogs treated per year in the randomly selected clinic located in northern part of Nairobi County.

s= Number of dogs treated per year in the randomly selected clinic located in southern part of Nairobi County.

w= Number of dogs treated per year in the randomly selected clinic located in Western part of Nairobi County.

e= Number of dogs treated per year in the randomly selected clinic located in Eastern part of Nairobi County.

400 = Calculated sample size

To avoid biasness, sample collection in veterinary clinic was carried out twice weekly (Monday and Thursday). These two days were selected to minimize chances of one dog being sampled twice. This is because it is a common practice for veterinarians in Nairobi to review treated cases within 72 hours. As such, any dog that was treated on Monday would have completed the review appointments by Wednesday and those treated on Thursday would have completed the review appointments by Saturday. The first dog presented in the clinic in the morning for treatment was sampled and thereafter every other third dog in order to minimize chances of sampling dogs from the same owner and therefore biasness. Sample collection in KSPCA was throughout where every dog admitted to the shelter facility was sampled. Samples were also collected during their scheduled clinics in low-income setups within the Nairobi County. Dogs from these low-income areas were free roaming since their owners did not house them and they were sampled during routine scheduled clinics that were offered by KSPCA in conjunction with Trap Neuter and Release (TNR), one health Kenya and the county government of Nairobi. These clinics included vaccination, deworming, neutering and treatment of all dogs in Nairobi County for free. However, to ensure systematic randomization of dogs, samples were collected from the first dog treated on the sampling day and thereafter every other third dog.

3.5. Sample collection

Four hundred faecal samples were collected between November 2018 to October 2019 from the 400 systematically randomly selected dogs in the selected veterinary clinics and KSPCA. Sample collection was distributed in both wet and dry seasons. Dry season in Nairobi was defined as clear sky and sunny days with an average temperature of 23^0C. These included January to February and July to September. The wet season was defined as the rainy months that occurred between March to June and October to December. There were 8 wet months which included March, April, May, June, October, November and December. On the other hand, 5 months were dry and they included January, February, July, August and September. However, the weather patterns were drastic during the entire sampling period with rains being experienced in months such as January and February that were otherwise known to be dry.

Approximately 5 grams of faecal samples were collected per rectum from each dog. The collected faecal samples were stored in appropriately labeled faecal containers, placed in the cool box and transported to the University of Nairobi, Department of Veterinary Pathology, Microbiology and Parasitology for processing. Samples were processed the same day or kept at 4^0C and processed within 48 hours.

3.6 Determination of *G. duodenalis* prevalence

To determine the prevalence, faecal samples were subjected to *Giardia* SNAP test kit (INDEX laboratories, USA) which is *Giardia* ELISA test specific for canine faeces. The test has a sensitivity of 95% and specificity of 99%. This test is a rapid enzyme immunoassay for the detection of *Giardia* antigen in faeces that is indicated by a blue color in the sample spot. The presence of the antigen indicated the dog has ingested *Giardia* cyst, may be actively infected or shedding cyst in faeces. Processing of faecal sample was done on fresh samples, previously frozen or stored at 4^0C. The sample must be stored in room temperature at 18 - 25^0C before processing. The kit has a conjugate/swab, which contain 0.7 ml of anti-*Giardia*

peroxidase solution and a snap device that contain 0.4 ml of wash solution and 0.6 ml of substrate solution.

3.6.1 Test procedure that detected *G. duodenalis* antigen by ELISA

The tube that covers the conjugate/swab device was pulled out and the entire swab tip coated with a thin layer of faecal material as shown in **Figure 3.2** below. Plastic valve stem inside the reagent bulb was broken at the neck by bending back and forth. Then the device swab tip was hold down and the bulb squeezed and released 3 times to pass the conjugate solution in the bulb to swab tip (**Figure 3.3)**.

The snap device was placed on a flat surface and then the tube from the conjugate/swab device removed. The swab was used as a pipette and 5 drops of sample/conjugate solution were dispensed into the sample well of the snap device being careful not to splash the content outside the sample well. The sample flowed across the result widow and reached the activation circle in approximately 30 - 60 seconds. After the sample first appeared in the activation circle, the activator button was pushed firmly until it flushed the device body as shown in **Figure 3.4**. Then the results were read after 8 minutes.

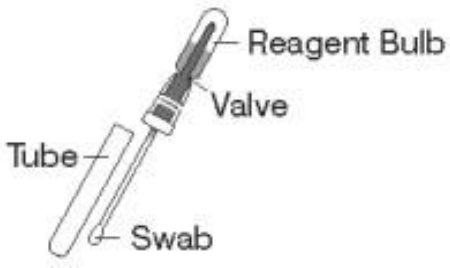

Figure 3.2: The conjugate device

Figure 3.3: The conjugate tube

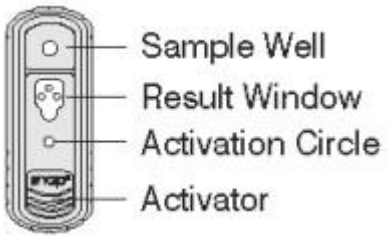

Figure 3.4: The snap device (IDEXX Laboratories, Inc 2018)

3.6.2 Interpretation of test results

The results were determined by reading the reaction spots in the result widow and then compared to the colour intensity of the sample spot to that of negative control spot. The negative control spots served as safeguard against false positive. The blue colour development on the positive spot indicates that test reagents are functional and help indicate that test was run properly as shown in the **Figure 3.5b**.

3.6.2.1 Negative test result

The result was negative for a sample spot if there was no colour on the sample spot and the negative control spot or colour on sample spot was equal to colour on negative control spot as shown in the Figure **3.5a** below.

3.6.2.2 Positive test result

A test was positive when the colour on the *Giardia* sample spot had a dark blue colour than the colour on the negative control as shown in the **Figure 3.5b**.

3.6.2.3 Invalid test result

The test result was considered invalid if the positive control spot did not develop colour, or when the colour on negative control spot was darker than on the sample spot.

Figure 3.5a: A negative test result (IDEXX Laboratories, inc 2018)

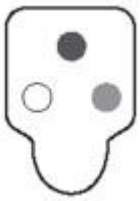

Figure 3.5b: A positive test result (IDEXX Laboratories, Inc 2018)

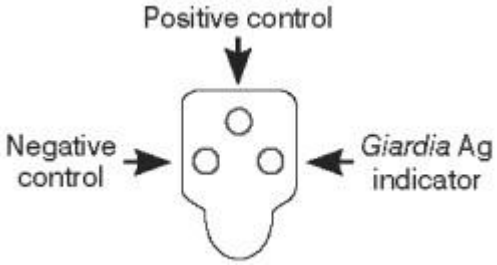

Figure 3.6: The result window (IDEXX Laboratories, Inc 2018)

3.7 Determination of potential risk factors for *G. duodenalis* infection

Data appertaining to potential risk factors for *Giardia* infection was obtained by administering a questionnaire (**Appendix 4**) to dog owner /care takers. These factors included age, ownership, breed, sex, neuter status, body condition score, faecal consistency score, clinical signs and duration, type of food, area of origin, season, nature of housing, method of housing(animal husbandry), purpose of keeping dog, vaccination status, co-infection and deworming status. The age of the dog was determined based on the information from the client and/or estimation by the veterinarian /trained research assistance using dentation features like teeth eruption, wearing and condition. Body condition score was classified in a Likert scale of 1-9 scale as shown in **Appendix 2.** Faecal consistency score was evaluated

using a protocol that utilizes pictorial appearance and subsequent rating in 1-5 Likert scale where 1 is diarrheic and 5 is dry and crumbly (**Appendix 3**).

3.8 Determination of geo-spatial distribution of *G. duodenalis*

The geospatial distribution of *Giardia* infection in dogs in Nairobi County was done using QGIS 2.0.1 software for mapping and SatScan for comparative analysis. The information related to dog owners physical address or the area where the dog originated was collected. The GPS co-ordinates for the physical address or area of origin was generated from Google map and entered in QGIS 2.0.1 software for further analysis. Nairobi County maps were obtained from government of Kenya GIS database.

3.9 Data Analysis

A database was created in Microsoft Excel 2010, where generated data was entered and then imported to Stata software for further statistical analysis such as descriptive summary statistics and computation of measures of association.

3.9.1 Determination of *G. duodenalis* prevalence

Overall true point prevalence of 95% confidence interval for *Giardia duodenalis* infection was calculated using descriptive statistics. To calculate the true point prevalence, the apparent prevalence hereby defined as percentage of positive samples was adjusted for the reported sensitivity and specificity of the *Giardia* ELISA test (n). The purpose of the adjustment was to factors in positivity results that were directly influenced by specificity (99%) and sensitivity (95%) of a test. The following formula was utilized:

$$n = \frac{\text{Apparent prevalence} + \text{specificity} - 1}{\text{Sensitivity} + \text{Specificity} - 1}$$

3.9.2 Potential risk factors

Multivariable logistic regression analysis was performed to identify risk factors associated with *G. duodenalis* infection. First, unconditional univariate analysis was carried out to

obtain probabilities of individual potential risk factors. Only those associations that were considered significant at a p value ≤ 0.2 were carried forward to the multivariable logistic regression model while assessing for confounding and interaction. Those variables that qualified for multivariable logistic regression analysis modelling were checked for collinearity through variance-covariance matrix of the estimators (VCE) and multicollinearity was found if Variance-covariance estimator was greater than five (5) for any pair (Dohoo *et al.*, 2009).

Statistical significance and credibility was therefore used to determine the collinear variables for the multivariable analysis. A manual backward stepwise fitting the logistic regression model was done by removing variables that were least significant and retaining variables that resulted in p value ≤ 0.05 in the final model. The odds ratios and confidence intervals for each of the significant risk factors were obtained from the model.

Confounding association was determined if removal of the predicable variables from the model modified the coefficients of other significant variables by 30%, in which case they were kept in the final model regardless of their P value.

3.9.3 Geo-spatial distribution

For spatial distribution, clustering of *Giardia* infection in dogs in Nairobi County was evaluated using Satscan software using QGIS to depict disease intensity per Km^2. Heat maps were generated at 6.5 Km bandwidth and 500m grid cells as reported by (Pfeiffer *et al.*, 2008). The software clustered the distribution of infection into thousand fold. From these thousand clusters of infection an antilog was used that determined the most significant clusters with high risk of infection at p value ≤ 0.05.

CHAPTER FOUR: RESULTS

4.1 Demographics characteristics

A total of 400 dogs were randomly sampled out of which 98 were from shelter and 302 were client owned dogs. Amongst the sampled dogs, 238 were male while 162 were females. The mean age and weight of the dogs was 41.3 ± 35.2 months and 25.1 ± 18.4 Kg, respectively. The most commonly sampled breeds of dogs were German shepherd (44.8%), followed by local breed (24.3%) and Rottweiler (7%). Most (83%) of the dogs were entire while the remaining 17% had been neutered. The mean body condition and faecal consistency score was 5.4 ± 1.0 and 3.3 ± 0.8, respectively.

The majority of the sampled dogs had up to date vaccination or deworming status. About 26 dogs exhibited diarrhea while 11 had both diarrhea and vomiting with just 7dogs vomiting only. The remaining (356) did not show any signs related to gastroenteritis. Most of the dogs were fed on commercial dog food while the rest were on homemade food. The dogs were served food and water on individual bowls and cleaning of the utensils was mostly done at least once daily. Most of the dogs were housed in groups while the rest were housed individually with majority of the dogs throughout the day and released at night while the rest were either kenneled or left out throughout. More than a half of the kennels were cleaned at least more than once daily.

4.2 Detection of *G. duodenalis* by ELISA test

G. duodenalis antigens were tested from all the 400 faecal samples collected by *Giardia* ELISA snap test (**Figure 4.1)**. Positivity was indicated by formation of two blue spots (**Figure 4.2a)** while the negativity was indicated by formation of a single blue spot (**Figure 4.2b)**.

Figure 4.1: *Giardia* ELISA snap test kit matched with the individual faecal samples

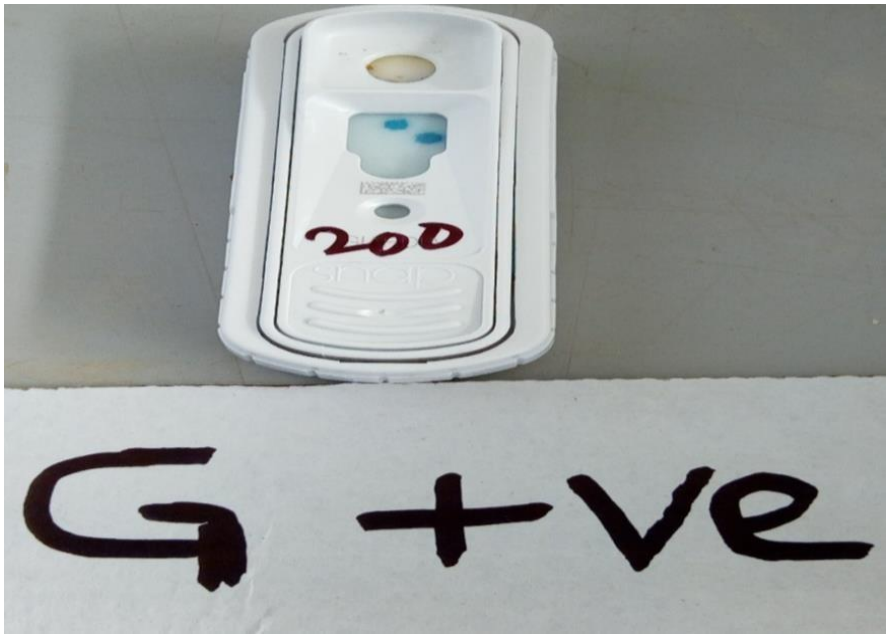

Figure 4.2a: A positive *G. duodenalis* test indicated by 2 blue spot

Figure 4.2b: A *G. duodenalis* negative test result indicated by a single blue spot

4.3 Prevalence of *G. duodenalis* in Nairobi County

4.3.1 Overall prevalence

Out of 400 samples tested with *Giardia* SNAP test, 89 faecal samples tested positive for *G. duodenalis* while 311 faecal samples tested negative. The overall apparent prevalence of *G. duodenalis* in Nairobi County, Kenya was 22.25% (**Figure 4.3)**. The calculated overall true point prevalence was 25% at 95% CI.

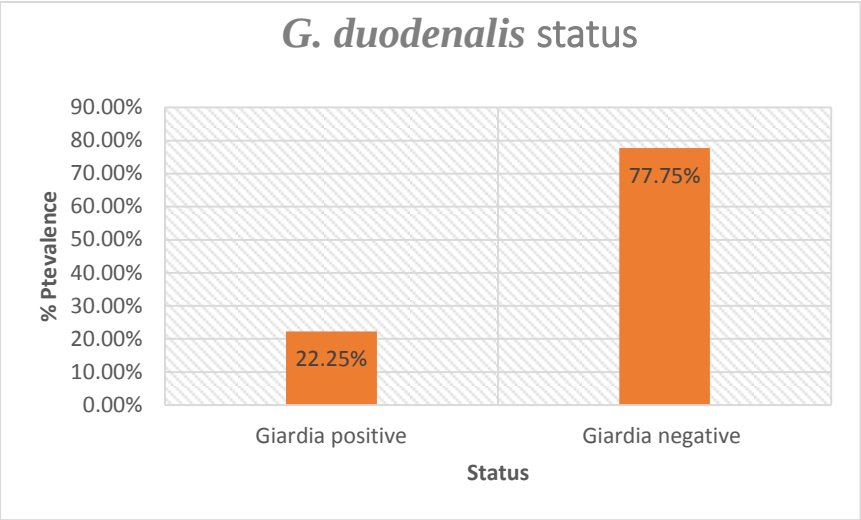

Figure 4.3: Overall apparent prevalence of *G. duodenalis* in Nairobi County

4.4 Analysis by risk factors

The infection of *G. duodenalis* infection was also calculated in each of the predicted risk factors to determine their effect in the occurrence of the infection.

4.4.1 Ownership

Dog ownership was a predicted factor for *G. duodenalis* infection in our study and from the 400 sample, 41/400 (10.25%) samples came from dogs sheltered after rescue by KSPCA, 96/400(24%) were free roaming dogs while 263/ 400 (66%) samples came from client owned dogs as shown in the **Table 4.1**. The prevalence of *G. duodenalis* infection was high in the free roaming dogs where 46/96 (47.91%) tested positive as compared to 7/41 (17.07%) KSPCA sheltered dogs and 36/263 (13.69%) from client owned dogs.

Table 4.1: Sample collected, respective prevalence of *G. duodenalis* by dog ownership

Ownership	No. Sampled	No. positive	Prevalence (%)
KSPCA Sheltered dogs	41	7	17.07
Free roaming	96	46	47.91
Client owned	263	36	13.69
Total/ Overall	**400**	**89**	**22.25**

4.4.2 Area of origin of the dog

The areas of origin were classified as high-income areas, middle income and low-income areas based human activity, level of sanitation and income. The majority of dogs came from high-income area of Nairobi county with 175/400 (43.75%) samples. These areas included Karen, Kilimani, Lang'ata, Lavington, Runda, Upperhill, Loresho, Parklands, Kitisuru, Westlands, and Gigiri. The middle-income areas followed with 131/400 (32.75%) samples. This came from Utawala, Kasarani, Roysambu, Imara Daima and Buruburu. The low-income areas had 94/400 (23.50%) samples that came from Kawangware, Kibera, Kangemi, Dandora, Kayole, Majengo, Eastleigh, Ruai and Mathare (**Table 4.2).**

The study showed that the prevalence of *G. duodenalis* infection was highest in dogs from low income areas with 35/94 (37.23%) testing positive followed by dogs from middle income areas where 34/133 (25.56%) tested positive and lowest in high income areas with 20/173 (11.56%) positive cases**.**

Table 4.2: Number of faecal samples collected and prevalence rates of *G. duodenalis* infection by areas of origin

Area of origin	No sampled	No. positive	Prevalence (%)
Low income area	94	35	37.23
Middle income area	133	34	25.56
High income area	173	20	11.56
Total/ Overall	**400**	**89**	**22.3**

4.4.3 Breed of the dog

The dogs sampled were classified as pure and mixed breed. There were 276/400 (69%) breed that included German Shepherd Dog, Rottweiler, Terrier, Mallinois, Boerboel, Labrador, Japanese spitz, JackRussell, St.Benard, Maltese, Bamese and Pomeranian. While mixed breed of dog were 124/400 (31%) as shown in **Table 4.3** below. This study showed that the prevalence rate of *G. duodenalis* infections was higher in mixed breed 37/124(29.84%) than in pure breeds of dogs at 62/276 (22.46%).

Table 4.3: The samples collected and prevalence of *G. duodenalis* infection in pure and mixed breed dogs

Breed	No sampled	No. positive	Prevalence (%)
Pure breed	276	62	22.46
Mixed breed	124	37	29.84
Total/ Overall	**400**	**89**	**22.25**

4.4.4 Age of the dog

Dogs between 0-12 months were considered young while dogs above 12 months considered adult based on dental formula, haricot, diet, body condition and behaviour. Therefore, 92/400 young dogs and 308/400 adult dogs were sampled (**Table 4.4)**. The study showed that the prevalence of *G. duodenalis* is higher in young dogs 46/92 (50%) testing positive for *G. duodenalis* and lower in adult dog 43/308 (13.96%) tested positive.

Table 4.4: Distribution of samples and prevalence rates of *G. duodenalis* infection by age

Age	No sampled	No positive	Prevalence (%)
Young	92	46	50
Adult	308	43	13.96
Total/ Overall	**400**	**89**	**22.25**

4.4.5 Sex of the dog

Out of 400 samples 162 were from female dogs while 238 were from males as shown in **Table 4.5**. The results of the study showed that the prevalence rate of *G. duodenalis* infection was high in male than female. About 59/232 (24.78%) male dogs tested positive while 30/162 (18.52%) females tested positive (**Table 4.5)**.

Table 4.5: Number of samples and prevalence rates of *G. duodenalis* infection by sex

Sex	No sampled	No. positive	Prevalence (%)
Male	238	59	24.78
Female	162	30	18.52
Total/ Overall	**400**	**89**	**22.25**

4.4.6 Season of the year

Season of the year was classified as wet and dry. Among the 400 samples, 70% of the samples collected in the wet season while 30% samples were collected in the dry season shown in **Table 4.6**. The prevalence of *G. duodenalis* infection was higher in wet season than the dry where 70/280 (25.0%) and 19/120 (15.83%) tested positive in respective seasons.

Table 4.6: Number of samples and prevalence rate of *G. duodenalis* by season

Season	No. sampled	No. positive	Prevalence (%)
Wet	280	70	25.0
Dry	120	19	15.83
Total/ Overall	400	89	22.25

4.4.7 Neuter status of the dog

Out of 400 samples, 332 sampled dogs were entire while 68 were neutered (**Table 4.7)**. The prevalence rate of *G. duodenalis* infection was higher in entire dogs with 77/332 (23.19%) testing positive than neutered dogs where 12/68 (17.65%) tested positive.

Table 4.7: Number of samples and prevalence rate of *G. duodenalis* infection by neutered dogs

Neuter status	No of samples	No. positive	Prevalence (%)
Entire	332	77	23.19
Neutered	68	12	17.65
Total/ Overall	**400**	**89**	**22.25**

4.4.8 Vaccination status of the dog

Vaccination in dogs in Kenya is usually against canine distemper, parvovirus, hepatitis, leptospirosis and rabies. The majority of the sampled dogs had an up to date vaccination status at 379/ 400. 12/400 dogs were vaccinated at least once while 9/400 had never been vaccinated **Table 4.8**. The prevalence rate of *G. duodenalis* infection was higher in dogs that were never vaccinated as compared to vaccinated dogs. 81/379 (21.37%) of the dogs with up to date vaccination status tested positive, 4/12 (33.33%) of the dog that were vaccinated at least once tested positive while 4/9 (44.44%) of the dogs that had never been vaccinated tested positive.

Table 4.8: Number of samples and prevalence rate *G. duodenalis* in different vaccination statuses of dogs

Vaccination status	No. Sampled	No. positive	Prevalence (%)
Up to date	379	81	21.37
At least once	12	4	33.33
Never	9	4	44.44
Total/ Overall	**400**	**89**	**22.25**

4.4.9 Deworming status of the dog

The routine deworming protocol is after every three months. Of the 400 sampled dogs, 26 had never been dewormed, 37 were dewormed once while 337 of them had an up to date deworming status **Table 4. 9**. The infection rate of *G. duodenalis* infection was highest in dogs dewormed at least once with 12/37 (32.43%) testing positive, followed by 8 /26 (30.76%) that had never been dewormed and lowest at 69/337 (20.47%) that had an up to date deworming status.

Table 4.9: Number of samples and prevalence of *G. duodenalis* in different deworming status of dogs

Deworming status	No. sampled	No. positive	Prevalence (%)
Never	26	8	30.76
At least once	37	12	32.43
Up to date	337	69	20.47
Total/ Overall	**400**	**89**	**22.25**

4.4.10 Clinical signs

From the 400 sampled dogs, the majority at 356 did not exhibit any clinical signs while 44 had clinical signs of diarrhea and vomiting that can be associated *Giardia* infection (**Table 4.10)**. The prevalence of *G. duodenalis* infection was higher in dogs with clinical signs of diarrhea and vomiting where 20/44 (44.45%) of them tested positive while those that had no clinical signs had a low infection 69/356 (19.38%).

Table 4.10: Clinical signs manifested and the prevalence rate

Clinical signs	No. sampled	No. positive	Prevalence (%)
No signs	356	69	19.38
Diarrhea and vomiting	44	20	45.45
Total/ Overall	**400**	**89**	**22.25**

4.4.11 Purpose of keeping the dog

The majority of dogs were kept for security purposes 295/400 followed by 91/400 dogs kept as pet and 14/400 for breeding (**Table 4.11**). The highest prevalence rate was observed in dogs kept as pet 26/91 (28.57%) and breeding dogs 4/14(28.57%). The lowest prevalence rate 59/295 (20%) was recorded in dogs kept for security purposes.

Table 4.11: Number of samples and prevalence rate of *G. duodenalis* in dogs by purpose of keeping

Purpose of keeping	No sampled	No. positive	Prevalence (%)
Security	295	59	20
Pet	91	26	28.57
Breeding	14	4	28.57
Total/ Overall	**400**	**89**	**22.25**

4.4.12 Type of food

From the study, the majority of the sampled dogs were fed on commercial feed only 135/400, followed by 118/400 fed on commercial and sometimes on homemade food while 95/400 were fed on homemade food only 95/400 and 52/400 dogs were fed on food served to the family shown in **Table 4.12**. The prevalence of *G. duodenalis* was found to be highest in dogs fed on food served to the family where 23/52 (44.23%) tested positive, followed by those fed on homemade food 25/95 (26.31%) and those dogs fed on a combination of

commercial and homemade food 29/118 (24.57%). The lowest 12/135 (8.89%) was recorded in dogs fed on commercial food only.

Table 4.12: Number of samples and prevalence rate of *G. duodenalis* infection based on the different type of food/feed/meals fed to the dog

Type of food	No sampled	No. positive	Prevalence (%)
Food served to the family	52	23	44.23
Homemade food	95	25	26.31
Commercial and homemade feed	118	29	24.57
Commercial feed	135	12	8.89
Total/ Overall	**400**	**89**	**22.25**

4.4.13 Nature of housing

The study shows that the majority of the dogs sampled 213/400 were housed in kennels during the day and freed at night, 97/400 of them were kept in kennels throughout while 90/400 of them were never housed as shown in **Table 4.13**. The result here indicated that the prevalence of *G. duodenalis* infection was highest in dogs that were never housed with 35/90 (38.89%) testing positive. This was followed by those that were housed in kennels throughout where 22/97 (22.68%) tested positive and lowest in those housed in kennels during the day and freed at night where 32/213 (15.02%) tested positive.

Table 4.13: Number of samples and prevalence rate *G. duodenalis* infection based on different nature of housing

Nature of housing	No sampled	No. positive	Prevalence (%)
Never housed	90	35	38.89
Housed in kennels throughout	97	22	22.68
Housed in Kennels during the day and freed at night	213	32	15.02
Total/ Overall	**400**	**89**	**22.25**

4.3.14 Animal husbandry

From the 311 housed dogs, 282 were housed in an individual kennel while 69 were housed grouped in one kennel as shown in the **Table 4.14**. The result demonstrated a higher positivity rate in dogs housed in-groups where 21/69(30.43%) tested positive compared to 68/242 (28.10%) dogs housed individually that tested positive.

Table 4.14: Number of samples collected and the positivity rate based on husbandry

Husbandry	No sampled	No positive	Prevalence (%)
Individual housed	242	68	28.10
Group housed	69	21	30.43
Total/ Overall	**311**	**89**	**22.25**

4.4.15 Co-infection

Other diseases/ or infection or parasitic infestation diagnosed alongside giardiasis at time of sampling were considered as co- infection with *G. duodenalis* infection. About 331/400 dogs had no co-infections. 47/400 dogs had other infections or diseases diagnosed during sample collection that included babesiosis, ehrlichiosis, parvovirus infection, canine distemper and tick fever. 22/400 had parasites infestation such as ticks, mites, fleas that were visible on the dogs fur and tapeworms that were extracted together during faecal sample collection that as

shown in **Table 4.15**. The prevalence of *G. duodenalis* was highest in dogs with other diagnosed infection as 14/47 (29.79%) dogs testing positive followed by dogs with no co-infection 72/331(21.75%). The lowest prevalence was 3/22(13.64%) in dogs with parasites infestation.

Table 4.15: Number of samples and prevalence of *G. duodenalis* on dogs with other co-infection

Co-infection	No sampled	No positive	Prevalence (%)
Co-infection with Parasites	22	3	13.64
Other infections	47	14	29.79
None	331	72	21.75
Total/ Overall	**400**	**89**	**22.25**

4.4.16 Faecal consistency score

The majority 345/400 of dogs sampled had faeces with normal consistency as 41/400 of them had loose/pasty or watery faecal consistency while 14/400 of them had hard or crumbled faeces (**Table 4.16)**. The results from the study revealed that the prevalence of *G. duodenalis* infection was highest in dogs with faeces of loose consistency where 19/41 (46.34%) tested positive. This was followed by those with hard faecal consistency where 3/14 (21.43%) tested positive. The lowest prevalence was in dogs with normal faecal consistency where 67/345 (19.42%) samples tested positive.

Table 4.16: Number of samples, the faecal consistency categories and prevalence rates of *Giardia* infections

Fecal consistency	No. sampled	No. positive	Prevalence (%)
Loose	41	19	46.34
Hard	14	3	21.43
Normal	345	67	19.42
Total/ Overall	**400**	**89**	**22.25**

4.4.17 Body condition score

Most, 385/400 of the sampled dogs had a normal body condition score (4 - 7 scale), 9/400 of them were obese (8 - 9 while 6/400 sampled dogs were thin and emaciated (1 - 3) as shown in **Table 4.17**. The prevalence of *G. duodenalis* infection was highest in thin and emaciated dogs where 3/6 (50%) tested positive, followed by 86/385(22.33%) of those with normal body condition. None of the obese dogs tested positive.

Table 4.17: Number of samples and prevalence rates of *G. duodenalis* based on body condition score

Body condition	No. Sampled	No positive	Prevalence (%)
Thin and emaciated	6	3	50
Normal	385	86	22.33
Obese	9	0	0
Total/ Overall	**400**	**89**	**22.25**

4.5 Determination of significant risk factors associated with *G. duodenalis* infection

The result of univariate logistic regression analysis for all the predicted potential risk factors of *G. duodenalis* infection is presented in **Table 4.18.** The analysis shows that 15 factors had univariable association ($P \leq 0.2$) value to the *G. duodenalis* occurrence, infection and transmission. These factors were considered for the inclusion criterion of multivariable analyses. These factors include area of origin, ownership, breed, age, sex, vaccination and deworming status, body condition score, fecal consistency score, clinical signs, season, purpose of keeping the dog, type of housing or the animal husbandry, type of food and nature of housing. **(Table: 4.19).**

Area of origin of the dog had a significant association ($p \leq 0.001$) to *G. duodenalis* infection with the risk of infection being high in dogs from low-income areas than from middle and high income areas. Dog ownership had a univariate significant association ($P \leq 0.001$) with occurrence and the infection of *G. duodenalis* infection with the high positivity rate seen in roaming dogs than in sheltered dogs and client owned dogs. While age of the dog also had a univariate significant association ($P \leq 0.001$) with *G. duodenalis* infection with the risk of infection in young dogs less than 12 months being higher than older dogs of more than 12 months.

Sex of the dog had a significant association ($P = 0.14$) with *G. duodenalis* infection. The risk of infection was higher in male dogs than female dogs. The body condition score of the dog also had a significant association ($P = 0.05$) to the occurrence of the *G. duodenalis* infection with thin and emaciated dogs being at risk of testing positive than the normal and obese dogs.

The faecal consistency score was an important risk factor shown to be significantly associated ($P = 0.2$) to the occurrence of *G. duodenalis* infection. Dogs with loose stool had a higher risk of positivity rate to the infection as compared to dog with non-loose stool. The

clinical signs manifested by the dog were also found to be important risk factors to *G. duodenalis* infection. Dogs with diarrhoea and/or vomiting showed a consistent and significant association (P = 0.001) to the *G. duodenalis* infection.

The type of housing had univariable association at p = 0.002 to the occurrence of *G. duodenalis* infection. Dogs that were housed in groups had a high risk of infection as compared to those dogs housed in individual kennels. The type of food offered to dogs was marginally associated (P < 0.001) with *G. duodenalis* infection. Dogs feed on food served to the family and homemade food had a high a risk of positivity to the infection than dog fed on purely commercial feed.

The nature of housing was another factor seen to have a significant association (p < 0.001) to *G. duodenalis* infection. Dogs that were never housed and dogs housed in kennel throughout had a high positivity rate as compared to dogs housed during the day and fled at night. Season also had a significant association (p = 0.152) to *G. duodenalis* infection with infection being more in wet than dry season of the year.

The breed of the dog was another important risk factor to *G. duodenalis* infection. Mixed breed of dogs had a high significant (P = 0.007) positivity rate to Giardia infection as compared to other breeds of dog. Vaccination status also showed a univariate association at (p = 0.18) with *G. duodenalis* infection with infection being more positive to dogs that had never been vaccinated as compared to dog that had been vaccinated. The deworming status of the dog too had a univariate association (p = 0.009) with *G. duodenalis* infection with a high positivity in dogs dewormed at least once.

In the multivariate logistic analysis, all variables found to have univariable associations to *G. duodenalis* infection were checked for collinearity in a backward stepwise multivariable

analysis. All risk factors with correlation coefficient >0.5 values were considered collinear and therefore dropped. Three variables with a ($p \leq 0.05$) were retained in the final model. These were age, season and nature of housing (**Table 4.20**).

Age had a strong and significant association to *G. duodenalis* infection. The probability that a young dogs less than 12 months was positive for *G. duodenalis* was 0.22 (95% CI: 0.18, 0.40) times than an adult dog above 12 months old. The infection was shown to decrease with increase in the age of dog. Season also had a strong association with *G. duodenalis* infection with the probability of a dog testing positive in wet season being 0.99(95% CL: 0.39, 2.56) times more than in dry season. Significant association to the infection was also seen in nature of housing. Dogs never kenneled were 3 times (95% CL: 1.64, 5.65) more likely to have *Giardia* infection compared to their counterparts kenneled throughout that were 1.61(95%: 0.82, 318) times likely to have *G. duodenalis* infection (**Table 4.20)**.

The results of the study demonstrated a significant positive interaction between age of the dog and the season of year. The occurrence of *G. duodenalis* infection was high in young dogs less or equal to 12 months irrespective of wet or dry season. However, the infection was only high in adult dogs > 12 months in wet season with probability of an adult dog testing positive during the wet season being 0.09 (95% CL: 0.01, 0.86) times as shown in the **Figure 4.4.**

Table 4.18: Univariate analysis of all factors predicted to be associated with the presence of *G. duodenalis* in dogs

Factor	Variable	No. of samples	No. positive (%)	P value
Ownership	KSPCA Sheltered KSPCA Free roaming dogs Client owned	41 96 263	7 (17.07) 46 (47.91) 36 (13.69)	0.001
Age	Young Adult	92 308	46 (50) 43 (13.96)	0.001
Sex	Female Male	162 238	30 (18.52) 59 (24.79)	0.14
Season	Wet Dry	280 120	70 (25.0) 19 (15.83)	0.152
Neuter status	Entire Neutered	332 68	77 (23.19) 12 (17.65)	0.32
Vaccination status	Never Up to date At least once	9 379 12	4 (44.44) 81 (21.37) 4 (33.33)	0.18
Deworming status	Never At least once Up to date	26 37 337	8 (30.76) 12 (32.43) 69 (20.47)	0.009
Purpose of keeping	Security Pet Breeding	295 91 14	59 (20) 26 (28.57) 4 (28.57)	0.116
Type of feed	Commercial & homemade Commercial only Homemade only Food served to the family	118 135 95 52	29 (24.57) 12 (8.89) 25 (26.31) 23 (44.23)	0.001
Fecal consistency score	Loose Normal Hard	41 345 14	19 (46.34) 67 (19.42) 3 (21.42)	0.2
Body condition score	Emaciated Normal Obese	6 385 9	3 (50) 86 (22.34) 0 (0)	0.05
Area of origin	High income Middle income Low income	173 94 94	20 (11.56) 34 (25.56) 34 (37.23)	0.001
Clinical signs	No signs Diarrhea and vomiting	356 44	69 (19.38) 20 (45.45)	0.01*
Nature of housing	Housed in Kennels throughout Never housed Housed during the day and fled at night	97 90 213	22 (22.68) 35 (38.89) 32 (15.02)	0.001*
Animal husbandry	Individual dog housing Grouped dogs housing	242 69	68 (28.10) 21 (30.43)	0.002
Breed	Mixed breed Pure breed	124 276	37 (29.84) 52 (18.84)	0.007
Co-infections	Parasites Other infections None	22 47 331	3 (13.64) 14 (29.78) 72 (21.75)	0.445*

Table 4.19: Description and univariable associations of predictor variables (p≤ 0.2) for the multivariable analysis

Variable	Category	No. sampled	No. positive %)	P value
Area of origin	Low Middle High	94 131 175	35 (37.23) 34 (25.95) 20 (11.56)	**0.001***
Breed	Mixed breed Pure breed	124 276	37(29.84) 52 (18.84)	**0.007**
Ownership	KSPCA Sheltered KSPCA free roaming Client owned	41 96 263	7 (17.07) 46 (47.91) 36 (13.69)	**0.001**
Age	Young Adult	92 308	46 (50) 43 (13.96)	**0.001**
Sex	Female Male	162 238	30 (18.51) 59 (24.79)	**0.14**
Vaccination status	Up to date At least once Never	379 12 9	81 (21.37) 4 (33.33) 4 (44.44)	**0.18***
Deworming status	Up to date At least once Never	337 37 26	69 (20.47) 12 (32.43) 8 (30.77)	**0.009***
Body condition score	Thin Normal Obese	6 385 9	3 (50) 86 (22.34) 0 (0)	**0.05***
Fecal consistency score	Loose Normal Hard	41 345 14	19 (46.34) 67 (19.42) 3 (21.43)	**0.2**
Clinical signs	No signs Diarrhea and vomiting	356 44	69 (19.38) 20 (45.45)	**0.01***
Season	Wet Dry	280 120	70 (25.) 14 (19.83)	**0.15**
Purpose of keeping dogs	Security Pet Breeding	295 91 14	59 (20) 26 (28.57) 4 (28.57)	**0.116***
Animal husbandry	Individual dog housing Grouped dog housing	242 69	68 (28.10) 21 (30.43)	**0.002**
Type of food	Commercial food Commercial & homemade food Homemade only Food served to the family	135 118 95 52	12 (8.89) 29 (24.57) 25 (26.32) 23 (44.23)	**0.001***
Nature of housing	Housed in Kennels throughout Never housed Housed in kennels during the day and freed at night	97 90 213	22 (22.68) 35 (38.89) 32 (15.02)	**0.001***

KEY: * used to indicate the overall p values of variable with more than 2 categories

Table 4.20: Final multivariable logistic regression analysis results to determine factors associated with Presence of *G. duodenalis*

Variable	Categories	Odd ratios	95%CI		P value
			LCI	UCI	
Age	Young	0.22	0.18	0.40	0.00
Season	Wet	0.99	0.39	2.56	0.99
Housing	Kenneled throughout Never Kenneled	1.61 3.04	0.82 1.64	3.18 5.65	0.17 0.00
Age ≠ Season	Adult≠ Wet	0.09	0.01	0.86	0.04

The interaction between age and season and their association to the occurrence of *G. duodenalis* infection are shown in Figure 4.4 below.

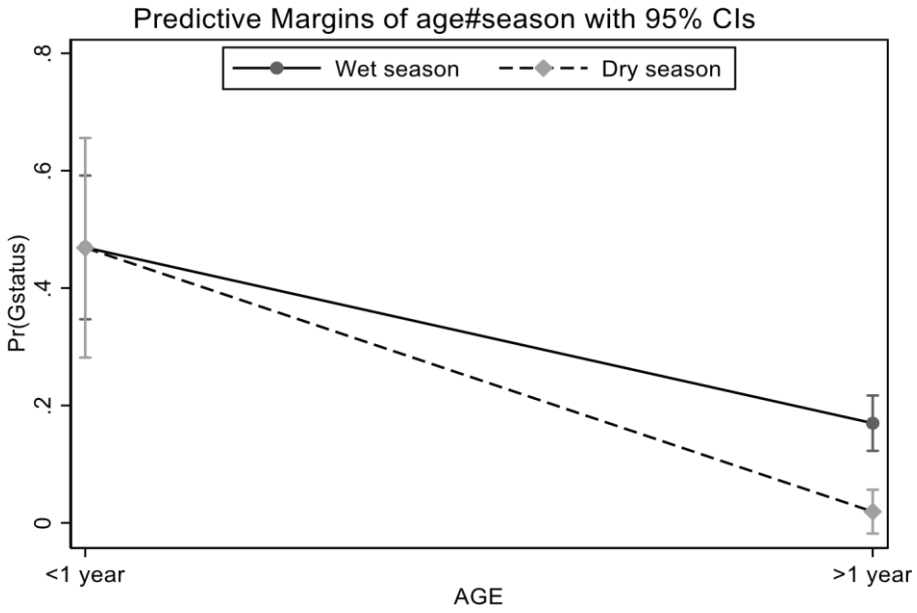

Figure 4.4: Interaction between age and season and their association to the occurrence of *G. duodenalis* infection.

The results further demonstrated a confounding association between age, season and nature of housing to the occurrence of *G. duodenalis* infection. Age on its own was found to have a direct association with occurrence of *G. duodenalis* infection. It also equally interacted with either season or nature of housing and as well as both season and nature of housing to predispose dogs to giardiasis. Equally, an interaction of season and nature of housing increased occurrence of giardiasis in dogs as shown in the **Figure 4.5** below.

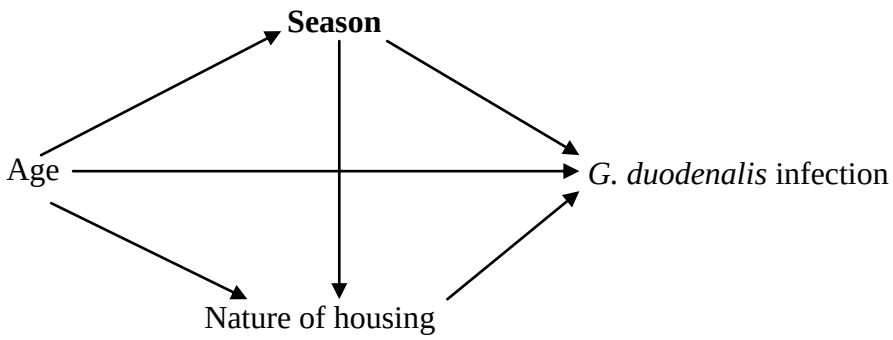

Figure 4.5: Confounding association between age, season and nature of housing to *G. duodenalis* infection

4.6 Spatial distribution *G. duodenalis* in dogs in Nairobi County

The results indicated that the *G. duodenalis* infection was distributed throughout the Nairobi County. However, the high risk of infection was seen in low-income slums of Kawangware, Kibera, Mathare, Dandora, Kangemi, Kayole, Ruai and Majengo as shown in F**igure 4.6** and **Figure 4.7**.These areas have a high population of human and free roaming dogs, also lack constant supply of clean water and have poor sanitation characterized by poor sewerage garbage disposal.

The results indicated that there were 5 clusters with high risk of infection, but only 2 showed statistical significant at $P \leq 0.05$. These clustering of infection were Kibera the largest slum in

Kenya and Ruai which is the sewerage collection point of Nairobi city **Figure 4.8** and **Figure 4.9** respectively**.**

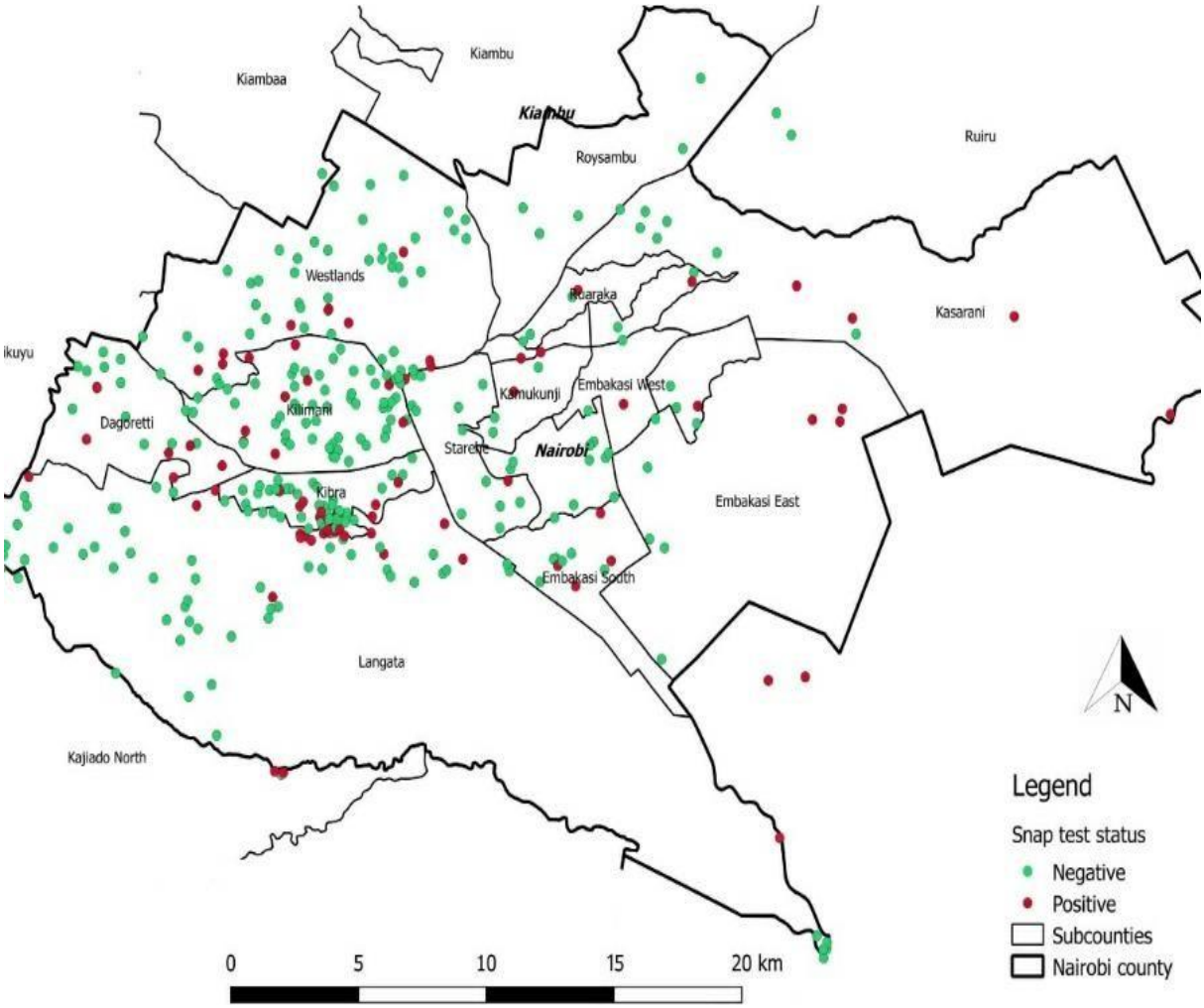

Figure 4.6: Photographic representation of point prevalence of canine *G. duodenalis* in Nairobi County, Kenya. The red spots indicated the origin of *Giardia* positive samples while the green spots indicated those of *Giardia* negative samples.

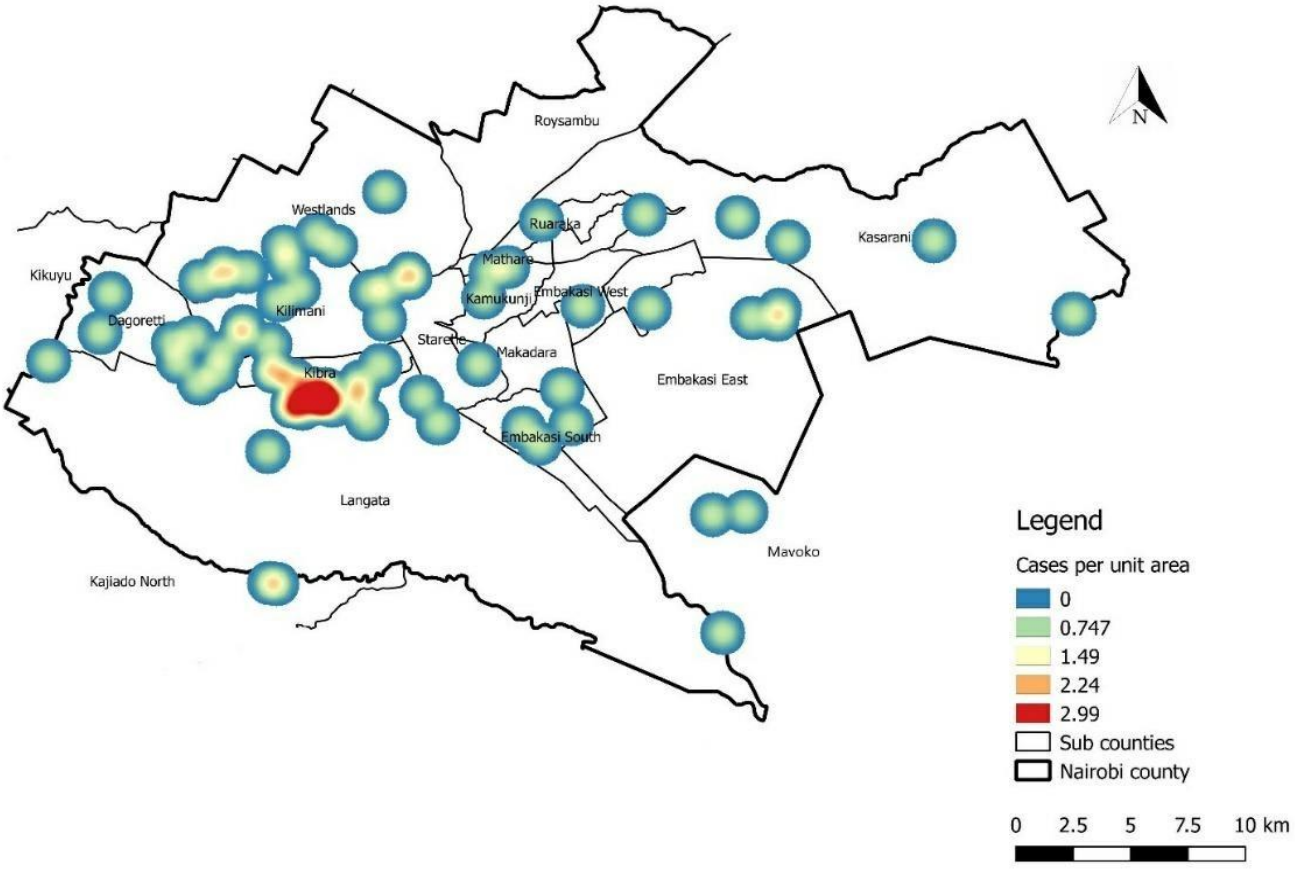

Figure 4.7: Photographic representation of hot points of canine *G. duodenalis* in Nairobi County, Kenya. The red and yellow colors indicated spots within Nairobi County where canine giardiasis was found to be high per unit area

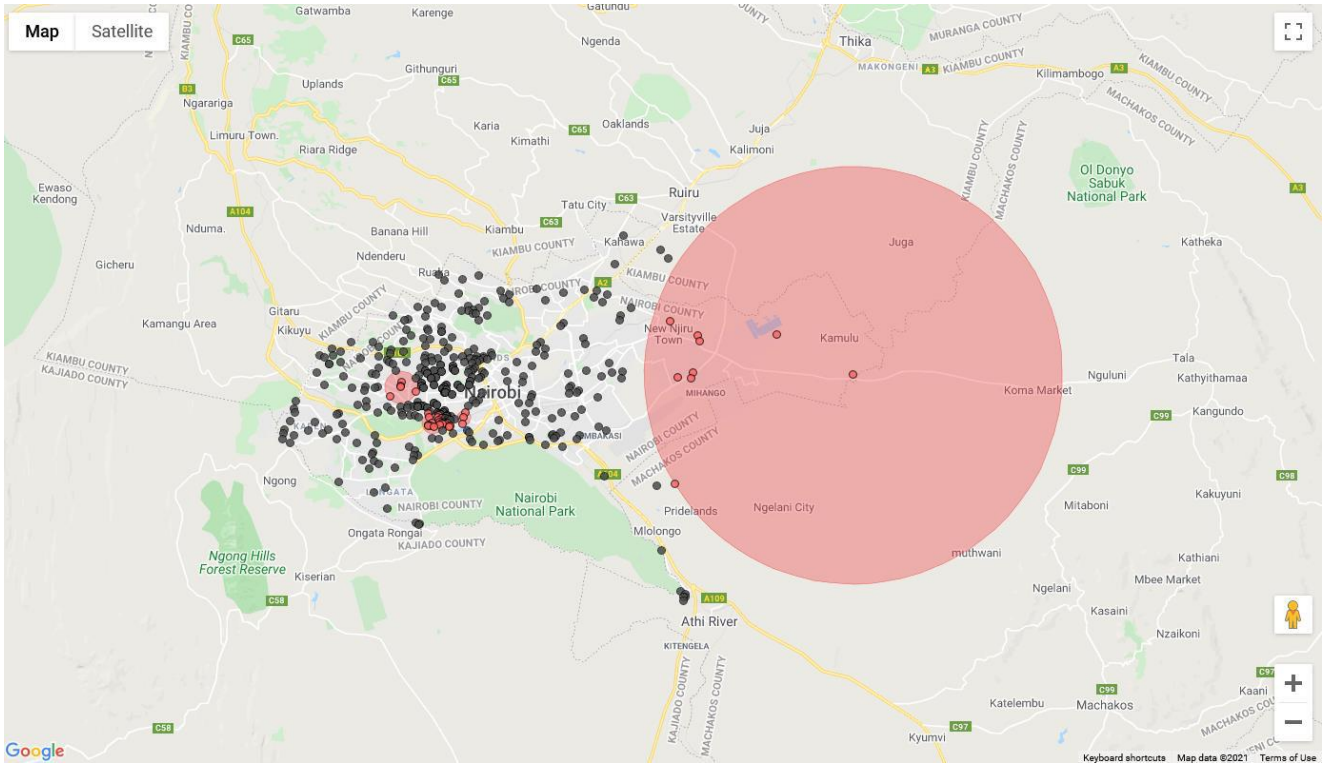

Figure 4.8: photographic representation of the 5 clusters that had the highest infection rate

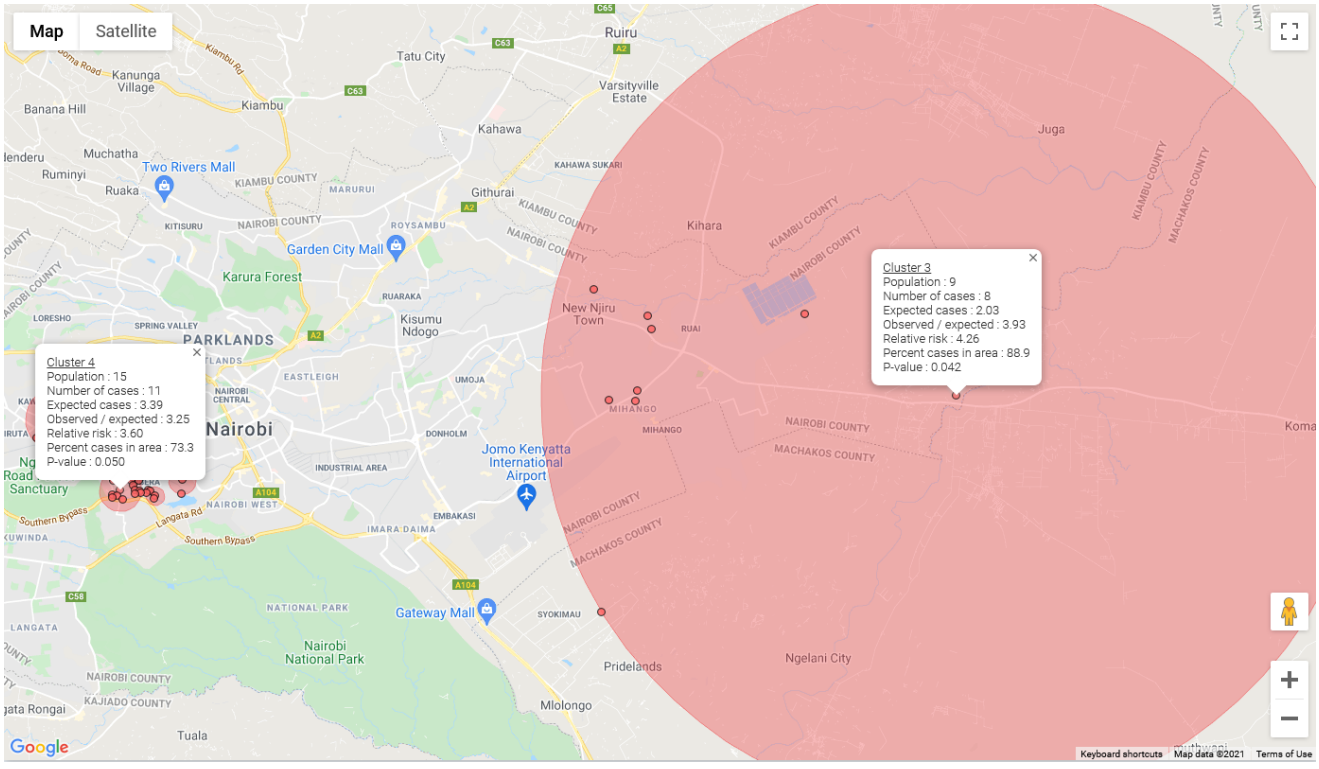

Figure 4.9: Photographic representation of the 2 significant clusters at P ≤0.05

CHAPTER FIVE: DISCUSSION, CONCLUSIONS AND RECCOMMENDATIONS

5.1 DISCUSSION

Giardia duodenalis is a common intestinal protozoan parasite that has been shown to infect both humans and animals. The prevalence rate varies depending on the method used in the detection and diagnoses and the risk factors of *G. duodenalis* worldwide.

5.1.1 Prevalence of *G.duodenalis* in Nairobi County, Kenya

Studies have shown that giardiasis is common in dogs with a prevalence range of 5-70% (Mircean *et al*., 2012). The prevalence rate of canine giardiasis in Nairobi County, Kenya was 22.25%. This was close to a prevalence of 26.3% reported in Italy 26.3% (Pipia *et al.,* 2014), 25% in Trinidad and Tobago (Mark-Carew *et al.,* 2013), 21% in central London (Upjohn *et al.,* 2010) and 25% in Mexico (Godínez-Galaz *et al*., 2019). This could be explained by the fact that Nairobi County has a warm and wet climatic condition, which could favour the life cycle of the parasite and eventual dispersion of cyst in the environment. In addition, the cyst form of the parasite is hard and stable in the environment and therefore can remain viable for long in the environment (Mossalanezhad *et al*., 2010). Additionally, the high population density of humans and the increasing number of free roaming dogs especially in poor set up areas within Nairobi County, contaminate the environment with cyst and therefore pose risk of infection to other dogs and human. These areas have poor sanitary condition, poor sewerage disposal and lack of clean water supply (Puebla *et al*., 2015).

The current reported prevalence in this study was however higher than the overall prevalence estimate of 15.2% reported in dogs worldwide (Godínez-Galaz *et al.,* 2019). It is also higher than what was recorded in China 14.3% (Qi *et al*., 2016), in Japan 15% (Itoh *et al.,* 2011), in USA 15.6% in symptomatic dogs (Carlin *et al.,* 2006), in Portugal 15.5%

(Neves *et al*., 2014), in Romania 8.5% (Mircean *et al.,* 2012), 13% in Canada (Olson *et al.,* 2010), in United States 0.44% in dogs with mixed clinical signs (Mohamed *et al*., 2013).

Although a prevalence of 22.3% was considered high in Kenya and in other Africa counties where prevalence rate ranges from 1.7% in Egypt (Ahmed *et al*., 2014), 5.6% in South Africa (Mukaratirwa and Singh, 2010) to 17.4% in Nigeria (Abubakar *et al.,* 2015, it is lower than a prevalence of 36.5% in Northern Spain (Adell-Aledon *et al.,* 2018), in Romania 34.6% (Mircean *et al*., 2012), in Colombia 39% (Pulido-Medellin *et al*., 2019), Mexico 42% (GodínezGalaz *et al*., 2019), India 40.8% in symptomatic dog (Sharma *et al.,* 2013), in France 41% (Grellet *et al*., 2014) and Canada 38% (Uehlinger *et al.,* 2013).

One possible explanation for the observed discrepancies in the prevalence is variations in faecal sampling technique and *Giardia* detection methods that were used. In the current study, samples were collected directly from the rectum and this could have resulted in an underestimated prevalence as *Giardia* cyst are usually shed intermittently in feces. This is in contrary to the study done by Panco-Macotela *et al*., (2005) that reported a very high prevalence where samples were collected directly from the intestine following euthanasia of stray dogs.

Another possible explanation could be the detection method. The current study utilized an ELISA based test that detects giardia antigen in fecal samples. The test has a reported sensitivity of 95% (95% CI 87%–98%) and a specificity of 99% (95% CI 96%–100%) (SNAP Giardia Antigen Test Kit product insert) and can therefore be able to detect a very small number of *Giardia* cyst in feces. On the contrary, a number of studies that reported lower prevalence utilized fecal flotation test and microscopy which is not a standardized method as concentration of cyst in faecal samples as well as experience in microscopic

identification of cyst can dramatically influence the sensitivity of the technique (Bouzid *et al*., 2015).

Although the sampling and detection method could have played a significant role in causing variation in prevalence between studies, epidemiological differences in study areas and the sampled dog populations cannot be overlooked. Case in point is reported tendency for higher infection rate in studies conducted in urban areas compared to rural areas (Mircean *et al*., 2012; Nguyen *et al*., 2018) and where stray dogs (Panco-Macotela *et al*., 2005; Becker *et al*., 2012), sick pets (Mahmud *et al*., 2014) and shelter dogs (Qi *et al*., 2016; Adell-Alledon *et al*., 2018) were sampled. The challenge with urban area is the dense populations and poor sanitation which can drive high infection rates of not only Giardia but other infectious diseases as well. Stray dogs and those from shelters are considered to be highly exposed to the risk of contracting giardiasis due to their scavenging behavior and lack of medical care. However, this explanation is subjective because some cosmopolitan areas (especially in developed countries) have proper infrastructure and good sanitation systems which would then mean minimal environmental contamination with *Giardia* cyst and hence low infection rate. In addition, management in shelters can also vary and impact on the prevalence of the disease amongst their resident dogs. This is argument is supported by evidence of some studies reporting a low prevalence rate despite being carried out in cosmopolitan areas and sampling being done from shelter population (Liao *et al*., 2020).

5.1.2 Risk factors associated with *G. duodenalis* infection

The age of the dog, season and nature of dog housing showed a significant association with occurrence of *G. duodenalis* infection. Young dogs were significantly ($p < 0.05$) infected with *G. duodenalis* than the older dogs with an infection rate of 13. 96%. This was similar to what was recorded in Romania at 12.1% and 47.1% in young dogs and at 6.4% and 28.3% in adult

dogs by faecal floatation and copro ELISA method respectively (Mircean *et al.,* 2012). It was also similar to what was reported in Vietnam at 12% in young and 4% in adult dogs (Nguyen *et al.,* 2018). These studies point out to the fact that puppies are more exposed to wide range of infection due to their lower level of immunity as compared to adult dogs (Choy *et al.,* 2014).

Additionally, younger dogs are frequently affected by giardiasis. They manifest a pronounced clinical signs that are observed within 2 weeks after ingestion of infectious cysts (Mravcova *et al*., 2019). In addition, the behavior of puppies' particularly biting and licking objects, which could be contaminated with *G. duodenalis* cyst, can be a contributing factor to the infection as shown by Mossalanezhad *et al*., (2010).

Therefore, young dogs are a high-risk group with high chances of shedding *cysts* in faeces within their first year but depending on the strain with the potential of spreading the infection to other dogs and humans. On the other hand, the study demonstrated that adult dogs were also infected with *G.duodenalis* but had a lower risk of developing infection unlike the young dogs. This can be explained by the fact that there is acquired immunity in adult dogs that may help control and/or reduce the severity of the infection (Adell-Aledón *et al.,* 2018).

At the same time, continuous exposure to infection in older dogs results in buildup of some protection with time. This is could be attributed to the fact that humoral immunity develops with age, thus a low infection rate in adult dogs (Nguyen *et al.,* 2018). Young dogs less than 12 months of age should therefore be separated from older dogs more than 12 months to help reduce the exposure of *G. duodenalis* infection among the dogs (Mark Carew *et al*., 2013).

This study demonstrated a significant higher ($p<0.05$) infection rate in the wet than in the dry season. This was in agreement with a high prevalence of 9.4% recorded in cold than 7% in warm season in Romania by floatation method and 44.9% in cold and 24.4% in wet season by copro ELISA (Mircean *et al*., 2012). The seasonal differences can be attributed to the fact that cysts can survive for a longer period in warm and moist environmental condition. This is because cysts are easily destroyed by desiccation in hot and dry climatic condition. They are also susceptible to ultraviolent light from direct sunlight (Centre for disease control and public health 2012). In addition, cyst can survive for long in the environment for up to 56 days in surface water. At 4^0C the cyst is viable for 90 days and 66 days at 12-22^0C (French agency for food, environmental and occupational health and safety, 2011**).** In addition, wet season provide a favourable prevailing environmental condition characterized by optimal humidity and temperature which make *Giardia* cyst viable and infective (Robertson and Lim, 2011).

There was a significant association ($p < 0.05$) between *G. duodenalis* infection and the nature of housing. This was similar to what was reported in Romania in dogs housed in kennels at 50% as tested by copro ELISA method (Mircean *et al.,* 2012) and 38% in stray dogs in a study in Colombia (Pulido-Medellin *et al*., 2019). This can be explained by the fact that, dogs that are never housed; are free to roam around and therefore more likely to be exposed to cyst contaminated soil, water and food in the environment. These roaming dogs end up ingesting these viable cyst in water and food and therefore get infected (Adell-Aledon *et al.,* 2018). Additionally, these roaming and stray dogs may access human feces and thus, more likely to ingest cyst in the faecal contaminated food and water (Horton *et al.,* 2019).

Kennel dogs on the other hand are known to developed coprophagic behavior due to stress from the confinement, which can lead to re-infection with *G. duodenalis*. This leads to

ingestion of cysts in faeces and therefore contributes to an important route of transmission in dogs (Uiterwijk *et al.,* 2019). This stress can also lead to impairment of immune responses in the intestine therefore becoming vulnerable to the infection (Pipia *et al*., 2014).

The high prevalence of *Giardia* infection in roaming and stray dogs was similar to what was recorded in dogs in Ireland at 67% (Horgan *et al*., 2020). This could be explained by the fact that most of dogs in slums are owned but they lack proper care in terms of housing, food and anti-parasitic treatment. This leads to dogs roaming around in search of food and water therefore exposing them to contaminated food, water and faeces and eventually predisposing them to poor health condition that subsequently increases the risk of *Giardia* infection. In addition, free roaming dogs has been demonstrated to play a significant role in spreading the *G. duodenalis* infection through dispersing and contaminating the environment with the infective cyst as they roam and hover around (Quadros *et al*., 2013).

However, the higher risk of infection in KSPCA sheltered dogs was in consistent with studies in Romania at 16.5% by floatation method and 47.7% by coproELISA method (Mircean *et al*., 2012), in Colombia at 17.3% (Pulido-Medellin *et al*., 2019) and in china at 13% recorded in sheltered dogs and 2% in household dogs (Liao *et al.,* 2020). In Italy, high rate was also recorded in sheltered dogs 35.5% (Pipia *et al.,* 2014). This could be explained by the fact that, these sheltered dogs were once free roaming in the streets and exposed to the cyst in the contaminated environment before being rescued and taken to the shelter facilities. Therefore, this could be a significant factor contributing to the high prevalence in shelter dogs (Liao *et al*., 2020).

In addition, dogs in these shelters experience stress from overcrowding or isolation, noise from the environment, changes in diet and limited physical activity. These stresses coupled with daily admission of other dogs from different origins, poor control of environmental

contamination from the infectious agent, and the long stay in these shelter facilities compromise the immune system and therefore makes the shelter environment conducive for infections. This make re-infection common and the newly admitted dogs can be exposed to infection (Raza *et al*., 2018).

The current study recorded a high infection rate in mixed breed than in pure breed of dogs. This was in agreement to what was reported in a similar study in Romania a high prevalence of 10% in mixed breed than 6.7% in purebred by floatation method and a prevalence of 35.1% in mixed breeds as compared to 33.6% in pure breed by copro ELISA method (Mircean *et al*., 2012). The restrictive indoor housing and existence for these pure breed that significantly lower risk of contracting giardiasis can explain this. This is unlike the crossbreed dogs that are more likely to be allowed to roam around (Pallant *et al*., 2015).

The current data also indicated a higher infection in male than in female dogs. This is in agreement with a previous study in Colombia that reported a high prevalence of 56% in male than 32% in female stray dogs (Pulido-Medellin *et al.,* 2019). However, reports from other part of the world reported a higher prevalence in female than males. In Romania, a higher prevalence of 39.8% in female than 31.2 % in male dogs by copro ELISA method was recorded. In addition, a prevalence of 9.9% in female and 7.4% in male dogs by faecal floatation method was reported in Romania (Mircean *et al*., 2012). In Colombia, a prevalence of 51% in female and 31% in male owned dogs was recorded (Pulido-Medellin *et al.,* 2019), in Vietnam 13% in female than 8% in male (Nguyen *et al*., 2018) and in Italy at 31.1% in female and 22.5% in male dogs (Pipia *et al*., 2014).

The gender based difference in prevalence could be attributed to the fact that, in regards to territorial habits, male dogs are known to have a wider area of operation than female dogs thus increasing the risk of infection. It could also be explained by the higher number of male

dogs presented to the clinic than the female. The high prevalence of *G. duodenalis* in female dogs in other studies could be attributed to the hormonal effect during the pro estrous, estrous, pregnancy and lactation period. The increased level of hormones during this period increases the risk of infection in the female dog (Bianciardi *et al*., 2004). Sex and pregnancy hormones in females and the regular period changes in hormonal levels have also been shown to interfere with the functions of cells of the innate immune system. Therefore, increases in the susceptibility of *G. duodenalis* infection (Roberts *et al.,* 2011). This study however, recommends further investigations to determine if there is association of *G. duodenalis* infections with sex of the dog and the effect of hormones in female and male dogs.

Entire dogs recorded a high positivity rate of *G. duodenalis* infection than neutered dogs. This was in consistent with the study in USA that reported a high prevalence rate of up to 25 - 30% in sexually intact dogs (Pulido-Medellin *et al*., 2019). It agreed to the records in Vietnam at 15% in entire and at 3% in neutered dogs (Nguyen *et al.,* 2018) and in Netherlands by Uiterwijk *et al.,* (2019) who demonstrated a high prevalence in intact male 25.4% than 18.4% neutered male and 37% high in entire female than 11% in neutered female. Male dogs are more aggressive and tend to dominate their female counterparts therefore comparative boldness. Moreover, entire male and female dogs can roam far and wide looking for a mate unlike the neutered exposing them to higher risk of contracting the infection (Starling *et al.,* 2019).

This study also showed a higher positivity rate in dogs with diarrhea and vomiting than in those with no signs. This was similar with the report in Iraq where a higher prevalence of 60% was recorded in cow with diarrhea due to *Giardia* than without 14.4% (AlhayalI *et al.,* 2020). In Iran, high prevalence (17.39%) was recorded in *Giardia* diarrheic cats as compared to non-diarrheic cats 0.79% (Mossalanezhad *et al.,* 2010). A similar study in Italy by Pipia *et*

al., (2014) also found correlation between diarrheic or pasty stool to presence of *Giardia* cysts in canine. This shows that diarrheal and/or vomiting can be important clinical signs that are suggestive of canine giardiasis (Laishram *et al*., 2012). This was in contrary to the study in Vietnam where the prevalence was to be high in dog with no clinical signs 18% than in dog with clinical sign 0%. This was attributed to the fact that *G. duodenalis* infection causes a chronic but intermittent diarrhea in the infected dogs hence difficult to correlate with the infection. This is because diarrhea in *Giardia* infection is self-limiting with most dogs being asymptomatic. These infected but asymptomatic dogs pose a major public health risk as the may act as a carrier or transmitter of *Giardia* infection to other dogs and human. On the other hand, clinically ill animals are likely to be taken for treatment and thus control the infection (Nguyen *et al*., 2018).

Faecal consistency score had a significant association to *G. duodenalis* infection where dogs with loose, watery or pasty stool had a higher prevalence of infection. This correlated with the study conducted in Italy where the prevalence in dog with diarrhea and or loose faeces was 34.2 % than in dogs with soft 26.3% and normal 18.3%. However, pasty faecal consistency has been reported to have twice as higher number of cyst as compared to diarrheic faeces (Pipia *et al.,* 2014). Uiterwijk *et al*., (2019) also recorded a prevalence of 40.8% in dogs with loose stool and 25.9% in dogs with non-loose stool.

However, Uiterwijk *et al.,* (2019) showed that there was a significant association with *G. duodenalis* positivity and loose stool as diarrhea or loose stool is a clinical sign indicative of giardiosis. This is because *Giardia* is known to cause acute, intermittent or chronic diarrhea in infected dogs which maybe asymptomatic or may develop nonspecific signs including severe enteritis, malodorous diarrhea (Laishram *et al*., 2012). *Giardia* should therefore not be investigated in dogs with diarrhea only (Pipia *et al.,* 2014).

The study also recorded higher infection rate in dogs that were never dewormed than those that were dewormed. This was similar to the report by Liao *et al*., (2020) where the infection rate was also higher in dogs that were not dewormed 15% than in dewormed dogs 5%. The lower infection rate in dog that were routinely dewormed could be explained by the fact that the helminth control by anthelmintics such Fenbendazole, Ivermectin, Praziquantel and pyrantel pamoate were also effective against protozoan infections, such as *Giardia*.

The study demonstrated a higher prevalence in dogs with poor body condition than in normal dogs. This can be attributed to management where by thin and emaciated dogs are not well taken care and lack good nutrition. Poor body condition has also been associated with high risk of infection and high prevalence of intestinal zoonotic parasite (Pulido-Medellin *et al.,* 2019).

It was also demonstrated that the infection was higher in dogs fed on home prepared food as compared to those fed on commercial feed. This correspond to the study in Midwest Brazil that also reported a higher prevalence of *G. duodenalis* infection in dogs fed on homemade food at 20% as compared to those fed on commercial food at 5.8%. This could be directed to poor hygiene during food preparation and serving that lead to contamination with the infective cyst and therefore ingestion of cyst in the contaminated food (Trevisan *et al.,* 2020). Additionally, the source of water for drinking, cleaning and food preparation could also be contaminated with the viable cyst (Choy *et al.*, 2014).

The current results also indicated a higher prevalence in dogs from low-income as compared to those from high-income areas. This correlated with a study in Vietnam that demonstrated that infection of *G. duodenalis* in dog had a significant variation in dogs from different area of origin (Nguyen *et al*., 2018**).** This could also be explained by the fact that infections in dogs reflect the practices of human activity in the area of origin in terms of level of

sanitation, waste and sewerage disposal. The sanitary levels are poor in low income areas and the parasite transmission from human to dogs is likely when the dogs consume human faeces or ingest *G. duodenalis* cyst in contaminate water and food (Horton *et al*., 2019**).** Also the ability to house their animals feed them and take them for veterinary care is minimal due to poverty caused by low income levels.

This study demonstrated a higher infection rate in dog with co-infections as compared to those without co-infections. This was similar to what was reported in Germany where infection was high in dogs and cats with other infections. This could be pointed to the fact that co-infection lowers the immune system therefore predisposing the animal to *G. duodenalis* infection (Pallant *et al*., 2015). *Cryptosporidium parvum* infection is also known to be associated with the occurrence of *G. duodenalis* infection (Liao *et al*., 2020).

The current data indicated that the prevalence of *G. duodenalis* infection was high in dogs that had never been vaccinated than vaccinated. This can be explained by management in that dogs that are routinely vaccinated against parvovirus, canine distemper, hepatitis and leptospirosis will help rule out gastroenteritis as a result of these diseases. Vaccination against these diseases also boasts the immune system of the dog. In addition, dogs that are routinely taken for vaccination are well take care by their owners where young dogs are kept indoors until all vaccination is completed unlike dogs that are never vaccinated therefore are at a lower risk of infection (Pallant *et al.*, 2015).

The current study demonstrated a higher infection in dogs kept as pet and for breeding purposes breeding compared to those kept for security. This can be explained by the fact that in breeding kennels, there are more young dogs whose immune system is not yet mature. This is in agreement to a prevalence of up to 100% reported in breeding kennels. Only about 10 %

positivity rate was detected in dogs kept under good hygiene conditions in breeding kennels as was highlighted by Raza *et al.,* (2018) and Mravcova *et al.,* (2019).

The high prevalence of *Giardia* infection in breeding dogs can also be explained by the effect of hormones in lactating bitches that has been reported to enhance the onset of cyst excretion. In addition, the large number of dogs in breeding kennels plus the high level of fecal contamination with Giardia cyst increases the stress levels and therefore increased ease of transmission (Bianciardi *et al*., 2004).

The high prevalence in pet dogs also corresponded to the report in Italy at 17% (Pipia *et al.,* 2014) and in Bangladesh 42.62% (Mahmud *et al*., 2014). A prevalence of 35.7% and 45.8% has also been reported in dogs kept as pet and for breeding respectively in Spain (Adell-Aledon *et al.,* 2018). This could be explained by the fact that pet dogs share the same compound with human. Therefore, infection and re-infection can occur when they ingest cysts from human and other canids in the same compound hence become reservoir of this *Giardia* parasite (Mahmud *et al*., 2014).

There was a higher prevalence rate in dogs housed in groups as compared to dogs housed in individual kennels. This is because dogs in groups are easily exposed to infective cysts. They also experience poor hygiene from the poor management practices due to lack of kennel cleaning. This makes them vulnerable to *G. duodenalis* infection**.** This is in agreement with surveys from previous studies that demonstrated a higher prevalence rate of *Giardia* in dogs in crowded kennels. This dense population enhances the ease of *G. duodenalis* transmission among the dogs (Mossalanezhad *et al.,* 2010). In addition, the exposure to high concentration of faecal waste and the diffuse contamination of cyst in dogs housed in group in a single kennels increases their risk of contraction the infection (Trevisan *et al.,* 2020). It has also

been shown than dog specific transmission cycle among dogs is favoured by high contact among dogs in groups (Mark -Carew *et al*., 2013).

5.1.2.1 Interaction between age and season to the occurrence of *G. duodenalis* infections

The present study demonstrated a positive interaction between age of the dog and season to *G. duodenalis* infection. The infection rate was high in young dogs irrespective it was wet and dry season while in adult dogs, the infection rate was only high in wet season. This can be attributed to the fact that climate changes can affect survival of the pathogen directly and it is spread in food, water and environment. This is by lengthening cyst survival in fomites, food and water, hastening the life cycle in the host, lower the infectious dose and increase the rate of cyst excretion (Lal *et al*., 2013).

In addition, during rainy season, the feces of the infected animals and the cystic contaminated soil is swept by flood to water bodies which are then used as source of water for drinking. There is also contamination of water sources by sewerages during rainy season due to flooding. This increases the risk of infection if the cyst is ingested in water (Noradilah *et al*., 2019; Lal *et al*., 2013). The infective stage of this parasite is hard and can survive up to 3 months in water (Choy *et al*., 2014). In addition, there has been a reported significant lags in *Giardia* infection with increased temperatures. Warm temperature on the other hand increases the infectivity of the cyst and *Giardia* host interaction opportunity. Increase in the relative humidity has also been reported to increase the *Giardia* infection (Lal *et al*., 2013).

5.1.3 Geospatial distribution of *G. duodenalis*

The study demonstrated that the *Giardia* infection is high in densely populated areas such as Kibera slum and its surrounding areas. Higher prevalence in dense populations maybe expected because of increased ease of transmission. This can be attributed to poor management where large numbers of dogs in these areas are not well kept and very few are

housed while majority roam around freely. Dogs in these areas also defecate indiscriminately disseminating parasites in the environment. On the other hand, housed dogs are normally taken out for a walk in gardens and public places where they are allowed to roam free for a while without a leash by their owner. Hence, they end up touching the contaminated environment with their snouts on the ground. These dogs are vulnerable and naïve and hence become infected with the infective cyst from the stray dogs **(**Mukaratirwa and Singh 2010).

This can also be pointed to the fact that low-income areas have the highest population density of humans, poor sanitation, lack constant supply of water and has poor sewerages disposal. At the same time, stray and owned dogs are all exposed to common factors that increase their risk of *G. duodenalis* infection. These factors are among; lack of access to clean water, poor hygiene and the exposure to cyst contaminated soil (Pulido-Medellin *et al.*, 2019). The high prevalence has also been linked to poverty in undeveloped countries and therefore unable to take care of their dogs and the lack of knowledge on molecular mechanisms for the disease (Savioli *et al*., 2006).

The high prevalence of *Giardia* infection in slums seen in the current study poses a significant public health risk and a major disease burden due to its propensity in causing major outbreaks and emergency to humans (Choy *et al.,* (2014; Puebla *et al.,* 2015). Previous studies have shown that dogs harbour either canine specific assemblages or the zoonotic assemblages. The cyst of the *Giardia* zoonotic assemblages A and B that are affect humans are also found in dogs and cat. Therefore, dogs as a carrier and reservoir of these assemblages can contaminate the environment posing a risk to humans and other dogs that move freely in the contaminated environment with poor sanitation and at the same time mixing freely with people.

5.2 CONCLUSIONS

1. The prevalence rate of *G. duodenalis* was found to be higher than what have been recorded in Africa and overall globally
2. The current study demonstrated that age, season and nature of dogs housing were significantly associated with *Giardia* cyst shedding
3. It was demonstrated that young dogs were significantly infected than adult dogs and the prevalence decreased with increasing age
4. The infection was found to be high in wet than in dry season. This showed that Nairobi County had a favourable climatic condition that favoured the spread of infection
5. Dog kenneled throughout and dogs that were never kenneled had a significant higher infection too
6. The geo-spatial distribution on the heat map shows that the prevalence of *G. duodenalis* infection in dogs is high in poor income areas which lack of basic sanitary condition and hence of important public health importance
7. The current study has demonstrated that dogs are important transmitter of *Giardia* and have an important role in the transmission of *G. duodenalis* infection

5.3 RECOMMENDATIONS

1. The control strategy for *G. duodenalis* should focus mainly on general education to dog owners or handlers in order create awareness and enlighten them on basic health and self-prevention measure on Giardia as a zoonotic parasitic infection to avoid being infected
2. Control and prevention measures on proper housing of dogs to keep them off from roaming around by the dog owners and provision of refugee by the KSPCA for stray, lost and unsheltered before they can be reclaimed rehomed or adopted
3. Government and non-governmental intervention policies and regulation in poor set up through provision of basic sanitation by proper garbage disposal and provision of clean

and adequate water. This will help minimize the risk of environmental contaminate by open sewerages that eventually contaminate food and water. Therefore, help decrease the Giardia infection in dogs and human and reduce the possibility of dog acting as reservoir host

4. KSPCA as the main shelter facilities in Kenya should follow guidelines suggested by the Society of Shelter Veterinarians to ensure the health of animals, working staff, visitors and new animal owners and prevent infection of newly admitted dogs in the shelters
5. The current study provided basis for further studies in other counties in Kenya in order to determine the overall epidemiological status that focuses on a wide sample area in order to determine if there are other significant factors associated with *G. duodenalis* infection apart from the ones demonstrated in the current study
6. Young dogs should be separated from the adult dogs to reduce the risk of exposure to
7. Giardia cyst from the infected adult dogs at young age
8. Further studies call for an important to perform a designed longitudinal study that will include appropriate sampling methods with combination of more than one diagnostic like microscopic examination, ELISA and PCR in order to estimate the real prevalence

REFERENCES

Abubakar, B.A., Maikai, B.V., Ajogi, I. and Otolorin, G.R., (2015). Prevalence of *Giardia* Cysts in Household Dog Faeces within Zaria Metropolis, Kaduna State, Nigeria and its Public Health Significance. Journal in Veterinary Advances, **5**(8): 1053-1057.

Adam, E. A, Yoder, J. S., Gould, L. H., Hlavsa, M.C., and Gargano, J.W., (2016). Giardiasis outbreaks in the United States. Epidemiology of Infectious disease journal, **144**(13): 2790-2801.

Adamska, M., Leońska-Duniec, A., Maciejewska, A., Sawczuk, M., Skotarczak, B., (2010). Comparison of efficiency of various DNA extraction methods from cysts of *Giardia intestinalis* measured by PCR and TaqMan real time PCR. Parasite**, 17:** 299-305.

Adell-Aledón, M., Köster, P. C., Lucio, A., Paula, P., Mingo, M.H, Sánchez-Thevenet, P., Dea-Ayuela, M.A., Carmena, D., (2018). Occurrence and molecular Epidemiology of *Giardia duodenalis* infection in dog populations in eastern Spain. .BMC Veterinary research**, 14**(26).

Ahmed, W.M., Mousa, W.M., Aboelhadid, S.M. and Tawfik, M.M., (2014). Prevalence of zoonotic and other gastrointestinal parasites in police and house dogs in Alexandria, Egypt. Veterinary World**, 7**: 275-280.

Alhayali, N. S, Alneema, M. S and Suleiman, G. E., (2020). Egyptian Journal of Veterinary Sciences. Egyptian journal of Veterinary Science**, 51**(3):381-390.

Becker, A.C., Rohen, M., Epe, C., Schnieder, T., (2012). Prevalence of endoparasites in stray and fostered dogs and cats in Northern Germany. Parasitology Research**,** **111**(2): 849-857.

Bianciardi, P., Papini, R., Giuliani, G., Cardini, G., (2004). Prevalence of *Giardia* antigen in stool samples from dogs and cats. Revue de Médicine Véterinaire, **155**, 8-9: 417-421.

Bouzid, M., Halai, K., Jeffreys, D., Hunter, P.R., (2015). The prevalence of *Giardia* infection in dogs and cats, a systematic review and meta-analysis of prevalence studies from stool samples. Veterinary Parasitology**, 207**: 181–202.

Bowman, D.D., Hendrix, C.M., Lindsay, D.S., (2002**).** Feline Clinical Parasitology. Ames, Iowa State University Press.

Carlin, E.C., Bowman, D.D., Scarlett, J.M., Garret, J. and Lorentzen, L., (2006). Prevalence of *Giardia* in symptomatic dogs and cats throughout the Unites States as determined by the IDEXX SNAP *Giardia* test. Veterinary Therapeutics**, 7(**3):199–206.

Centre for food security and public health, (2012). *Giardiasis*. Institute for international cooperation in animal biologics. College of veterinary medicine, Iowa State University.

Chavez-Munguia, B., Omana-Molina, M., Gonzalez-Lazaro, M., Gonzales-Robles, A., Cedillo Rivera, R., Bonilla, P., Martinez-Palomo, A., (2007). Ultrastructure of cyst differentiation in parasitic protozoa. Parasitology research**, 100** (6):1169-1175.

Choy, S.H., Al-Mekhlafi, H.M., Mahdy, M.A., Nasr, N. N., Sulaiman, M., Lim, Y. A., Surin, J., (2014). Prevalence and associated risk factors of Giardia infection among indigenous communities in rural Malaysia. Scientific Report, **4**(1):6909.

Dohoo, I., Martin W., and Stryhn H. (2003**).** Veterinary Epidemiologic Research**.** Sixth edition. AVC Inc., Canada pp 39-41.

Fantinatti, M., (2019). Zoonotic potential of *Giardia lamblia* and control of giardiasis Insights. Veterinary Science, **3:** 001-004. DOI: 10.29328/journal.ivs.1001013.

Feng, Y and Xiao, L, (2011**).** Zoonotic Potential and Molecular Epidemiology of *Giardia* Species and Giardiasis. Clinical microbiology reviews, pp110–140.

Ferreira, F.S., Bandeira, M.B.S.S.A.R., Constantino, F.S.C.A.C., Fonseca, C.D.T.A., Gomes M.J, Rodrigues L.M.R, Atouguia S.M.L.J, and Centeno-Lima F.A.C.S., (2013). Molecular and Clinical Characterization of *Giardia duodenalis* Infection in Preschool Children from Lisbon, Portugal**.** Journal of Parasitology Research**,** Volume 2013, Article ID 252971, **http://dx.doi.org/10.1155/2013/252971.**

French agency for food, environmental and occupational health and safety, (2011). *Data sheet on foodborne biological hazards*

Godínez-Galaz, E.M., Veyna-Salazar, N.P, Olvera-Ramírez, A, Razo, C.A.P, Bernal Reynaga, R., Alarcón, G.J.C., (2019). Prevalence and Zoonotic Potential of Giardia intestinalis in Dogs of the Central Region Mexico. Animals, **9 (**6): 325; https://doi.org/10.3390/ani9060325.

Gracenea, M., Gómez, M.S and Torres, J., (2009). Prevalence of intestinal parasites in shelter dogs and cats in the metropolitan area of Barcelona (Spain). Acta Parasitologica, **54**(1), 73–77

Hawash. Y., (2014). DNA Extraction from Protozoan Oocysts/Cysts in Feces for Diagnostic PCR. Korean Journal of Parasitology**, 52(**3): 263-271.

Horton, B., Bridle, H., Alexander, C.L., Katzer, F., (2019). *G. duodenalis* in the UK: Current knowledge of risk factors and public health implications. Journal of parasitology, **146** (4):413-424.

Horgan, E.R., O'Connor, L.M., Gately, .R.E., McNamara, A.B., Osborne, C.R., Morris. D.C., (2020). Prevalence of Human Specific Giardia lamblia in Irish Sheltered Canines: An Unknown Risk Factor for Human Infection. Journal of Veterinary Medicine and Public Health**, 1**(2): 48-52.

Grellet, A., Chastant-Maillard, S., Robin, C., Feugier, A., Boogaerts, C., Boucraut-Baralon, C., Grandjean, D., Polack, B., (2014). Risk factors of weaning diarrhea in puppies housed in breeding kennels. Journal of Preventive Veterinary Medicine**, 117**: 260–265.

Itoh, N., Kanai, .K, Tominaga, H., Kawamata, J., Kaneshima, T., Chikazawa, S., Hori, Y., Hoshi, F., Higuchi, S., (2011). *Giardia* and other intestinal parasites in dogs from veterinary clinics in Japan. Journal of Parasitology Research, **109**:253–256.

Kanyari, P.W.N., Kagira, J. M., Mhoma, J.R.L., (2010). Prevalence of endoparasites in cattle within urban and peri-urban areas of Lake Victoria Basin, Kenya with special reference to zoonotic potential. Scientific Parasitology, **11**(4):171-178.

Kwannan, N., Mathirut, M., Peerapan, T., Ram, R., Tawee, N., and Saovanee, L., (2007). Evaluation of the Sensitivities of DNA Extraction and PCR Methods for Detection of *Giardia duodenalis* in Stool Specimen**s.** Journal of clinical microbiology**, 45** (2):581–583.

Lalle, M., Jimenez-Cardosa, E., Cacciò, S.M. and Pozio, E., (2005)**.** Genotyping of *Giardia duodenalis* from humans and dogs from Mexico using a beta-giardin nested polymerase chain reaction assay. Journal of Parasitology, **91**(1):203–205.

Leclerc, H., Schwartzbrod L., and Dei-Cas, E., (2002). Microbial agents associated waterborne diseases. Critical Review in Microbiology, **28(**4): 371-409.

Laishram, S., Kang, G. & Ajjampur, S.S.R., (2012). Giardiasis: A review on assemblage distribution and epidemiology in India. Indian Journal of Gastroenterology**, 31**(1):3–12.

Leonhard, S., Pfister, K., Beelitz, P., Wielinga, C., Thompson, R.C.A., (2007)**.** The molecular characterization of *Giardia* from dogs in Germany. Veterinary Parasitology, **150**: 33-38.

Liao, S., Lin, X., Sun, Y., Qi1, N., Lv, M., Wu, C., Li, J., Hu, J., Yu, L., Cai, H., Xiao, W., Sun, M., Li, G., (2020). Occurrence and genotypes of *Cryptosporidium* spp., *Giardia duodenalis*, and *Blastocystis* sp.in household, shelter, breeding, and pet market dogs in Guangzhou, southern China**.** Scientific Reports**, 10:**17736 **https://doi.org/10.1038/s41598-020-74299-z.**

Lal, A., Baker, G. M., Hales, S., French, P.N., (2013). Potential effects of global environmental changes on cryptosporidiosis and giardiasis transmission. Trends in Parasitology, **29** (2)

Mahmud, M.A.A., Belal, S. M. S. H and Uddin, F.M. J., (2014). Prevalence of protozoan diseases in pet dogs at District veterinary hospital, Sirajganj, Bangladesh. Bangladesh Journal of Veterinary Medicine, **12** (2): 191-196.

Mark-Carew, M.P., Adesiyun, A.A.., Basu, A., Georges, K.A., Pierre, T., Tilitz, S., Wadea, S.E., Mohammed, H.O., (2013). Characterization of *G duodenalis* infections in dogs in Trinidad and Tobago. Veterinary Parasitology, **196**; 199– 202.

Mbae, C., Mulinge, E., Guleid, F., Wainaina, J., Waruru, A., Njiru, K. and Kariuki, S. (2016). Molecular Characterization of *Giardia duodenalis* in Children in Kenya. BMC Infectious Diseases**, 16**:135.

Malekifard, F. and Ahmadpour, M., (2018) Molecular detection and identification of *Giardia duodenalis* in cattle of Urmia, northwest of Iran. Veterinary Research Forum, 9 **(1***):* **81 – 85.**

Mircean, V., Györke, A., Cozma, V., **(2012).** Prevalence and risk factors of *G. duodenalis* in dogs from Romania**.** Veterinary Parasitology, **184:**325– 329.

Mosallanejad, B., Avizeh, R., Razi Jalali, M.H., Alborzi, A.R., (2010). Prevalence of *Giardia duodenalis* Infection in Household Cats of Ahvaz District, South-West of Iran *Iranian.* Journal of Parasitology**, 5** (3): 27-34.

Mohamed, A.S, Glickman, L.T, Camp, J.W, Lund, E, Moore, G.E., (2013). Prevalence and risk factors for Giardia spp. Infection in a large national sample of pet dogs visiting veterinary hospitals in the United States (2003–2009). Journal of Veterinary Parasitology, **195**(1-2):35-41.

Moron-Soto, M., Gutierrez, L., Sumano, H., Tapia. G., Alcala-Canto, Y., (2017). Efficacy of Nitazoxanide to treat natural Giardia infections in dogs. Parasites and Vectors**, 10**:52. DOI 10.1186/s13071-017-1998-7.

Mravcová, K., Štrkolcová, G., Goldová, M., (2019)**.** The prevalence and assemblages of *Giardia duodenalis* in dogs: a systematic review in Europe. Folia Veterinaria, **63(**4): 38-45.

Mukaratirwa, S. and Singh, V. P., (2010)**.** Prevalence of Gastrointestinal Parasites of Stray Dogs Impounded by the Society for the Prevention of Cruelty to Animals (SPCA), Durban and Coast, South Africa. Journal of the South African Veterinary association, **81**:123–125**.**

Neves, D., Lobo, L., Simões, P. B, Cardoso, L., (2014). Frequency of intestinal parasites in pet dogs from an urban area (Greater Oporto, northern Portugal). Journal of Veterinary Parasitology, **200** (3–4):295-298.

Nguyen, S.T., Fukuda, Y., Nguyen, D.T., Dao, H.T., Lei, D.Q., Bui, K.L., Tada, C and Nakai Y., (2018). Prevalence, genotyping and risk factors of *Giardia duodenalis* from dogs in Vietnam**.** Journal of Veterinary Medicine Sciences**, 80**(1): 92–97**.**

Noradilah, S.A., Moktar, N., Lee, L. L., Salleh, F. M., Anuar, T. S., (2019). Impact of seasonality on the prevalence and risk factors of *Giardia lamblia* infections among the aborigines. Asian Pacific Journal of Tropical Medicine, **12**(7): 315-320.

Olson, M.E., Leonard, N. J., Strout. J., (2010). Prevalence and diagnosis of Giardia infection in dogs and cats using a fecal antigen test and fecal smear. Canadian Veterinary Journal**, 51**(6):640-642.

Pallant, L., Barutzki, D., Schaper, R., Thompson, R. C. A., (2015). The epidemiology of infections with Giardia species and genotypes in well cared for dogs and cats in Germany. Parasites and Vectors, **8**:2.

Palmer, C.S, Traub, R.J, Robertson, I.D, Devlin, G., Rees, R., Thompson, R.C.A., (2008**).** Determining the zoonotic significance of Giardia and Cryptosporidium in Australian dogs and cats. Veterinary Parasitology**, 154**:142–147.

Pfeiffer, D., Robinson, T, Stevenson, M., Stevens, K., Rogers, D. and Clements, A., **(2008).** Spatial Analysis in Epidemiology**.** Oxford: Oxford University Press**.**

Pipia, P. A., Varcasia, A., Tamponi, C., Sanna, G., Soda, M., Paoletti, B., Traversa, D., Scala, A., (2014**).** Canine Giardiosis in Sardinia Island, Italy: prevalence, molecular characterization, and risk factors. Journal of Infectious disease in Developing Countries, **8** (5):655-660.

Ponce-Macotela, M., Peralta-Abarca, G.E., Martínez-Gordillo, M.N., (2005). *Giardia intestinalis* and other zoonotic parasites: prevalence in adult dogs from the southern part of Mexico City. Veterinary Parasitology, **131**(1-2): 1- 4**.**

Puebla, L.E.J., Núñez, F.A., Silva, I. M., Rivero, L.R., González, M.M, Sutil, Y.M., Valdés, L.A., Millán, I.A., Müller, N., (2015). Molecular Characterization and Risk Factors of *Giardia duodenalis* among School Children from La Habana, Cuba. Journal of Parasitology Research, Volume 2015, Article ID 378643.

Pulido-Medellín, M.O., Giraldo-Forero, J.C., Chavarro-Tulcán, G. I., (2019). Prevalence and risk factors of giardia spp. from free living and owned dogs in Tunja-Boyacá, Colombia. Bulgarian Journal of Veterinary Medicine, **22**(3):337–343.

Qi, M., Dong H., Wang, R., Li J., Zhao, J., Zhang, L., Luo, J., (2016). Infection rate and genetic diversity of *Giardia duodenalis* in pet and stray dogs in Henan Province, China. Parasitology International, **65**: 159–162.

Quadros, R.M.D., Weiss, P.H. E., Marques, S.M.T., Miletti, L.C., (2016). Potential cross contamination of similar *Giardia duodenalis* assemblage in children and pet dogs in southern Brazil, as determined by PCR-RFLP. Journal of Sao Paulo institute of tropical medicine, **58**:66.

Quadros, R. M., Weiss, P. H. E., Ezequiel, G. W., Tamanho, R. B., Lepo, G., Silva, M. R., Silva Junior, C R. J., Araujo, F. A. P., Miletti, L. C., (2013). Prevalence of *Giardia duodenalis* among dogs seized by the Center for Control of Zoonoses (CCZ) of the city of Lages, Santa Catarina, Brazil**.** Health, **5**(1): 119-124**.**

Raza, A., Rand, J., Qamar, G.A., Jabbar, A., Kopp, S., (2018). Gastrointestinal Parasites in Shelter Dogs: Occurrence, Pathology, Treatment and Risk to Shelter Workers. Animals, **8:** 108**.**

Roberts, C.W., Walker, W. and Alexander, J., (2011). Sex-Associated Hormones and Immunity to Protozoan Parasites. Clinical Microbiology Review, **14**(3): 476–488.

Robertson, L. J., Lim,Y.A.L., 2011. Waterborne and Environmentally-Borne Giardiasis. In: Luján, H.D., Svärd, S. (Eds.), Giardia. Springer Wien New York, 29–69.

Ropolo, A. S., Saura, A., Carranza, P. G., & Lujan, H. D., (2005). Identification of variantspecific surface proteins in *Giardia muris* trophozoites. Infection and immunity, **73**(8): 5208–5211. https://doi.org/10.1128/IAI.73.8.5208-5211.2005**.**

Savioli, L., Smith, H., Thompson, A., (2006). Giardia and Cryptosporidium join the 'Neglected Diseases Initiative'. Trends Parasitology, **22**(5): 203-208.

Sharma, S., Katoch, R., Yadav, A., Godara, R., (2013). Giardia prevalence in pet dogs in Jammu, India. Journal of Veterinary Practitioner, **14** (1):181-182.

Starling, M., Fawcett, A., Wilson, B., Serpell, J., McGreevy, P., (2019). Behavioral risks in female dogs with minimal lifetime exposure to gonadal hormones. PLOS ONE, **14**(12): e0223709. https://doi.org/10.1371/journal.pone.0223709.

Tangtrongsup, S., (2013). *Molecular epidemiology of giardia and cryptosporidium in dogs and cats in Chiang Mai, Thailand.* PHD Thesis. Colorado State University.

Trevisan, Y. P. A., Almeida, A. B. P. F., Nakazato, L., Pacheco, T. A., Souza, J. I., Canei, D. H., Pereira, M. E., Maia, M. O., Pacheco, R. C., Sousa, V.R.F., (2020). Frequency of *Giardia duodenalis* infection and its genetic variability in dogs in Cuiabá, Midwest Brazil. Journal of Infection in Developing Countries, **4**(12):1431-1436. doi:10.3855/jidc.13095.

WHO and UNICEF (2008), Joint Monitoring Programme for Water Supply and Sanitation 2008: 1-58

Uehlinger, F.D., Greenwood, S.J., McClure, J.T., Conboy, G, O.,'Handley, R., Barkema, H.W., (2013)**.** Zoonotic potential of *Giardia duodenalis* and Cryptosporidium spp. and prevalence of intestinal parasites in young dogs from different populations on Prince Edward Island, Canada. Journal of veterinary parasitology, **196**(3-4):509-514.

Uiterwijk, M., Nijsse, R., Kooyman, F. N. J., Wagenaar, J.A., Mughini‐Gras, L and Ploeger, H.W., (2019). Host factors associated with *Giardia duodenalis* infection in dogs across multiple diagnostic tests. Parasites Vectors, **12**:556.

Upjohn, M., Cobb, C., Monger, J., Geurden, T., Claerebout, E., Fox, M., (2010). Prevalence, molecular typing and risk factor analysis for *Giardia duodenalis* infections in dogs in a central London rescue shelter. Veterinary Parasitology, **172:**341-346**.**

Zhang, Y., Zhong, Z., Deng. L., Wang, M., Li, W., Gong, C., Fu. H., Cao, S., Shi, X., Wu, K., Peng, G., (2017). Detection and multilocus genotyping of Giardia duodenalis in dogs in Sichuan province, China. Parasite, **24:** 31.

APPENDICES

APPENDIX 1: WELFARE AND ETHICAL APPROVAL

UNIVERSITY OF NAIROBI
FACULTY OF VETERINARY MEDICINE
DEPARTMENT OF VETERINARY ANATOMY AND PHYSIOLOGY

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Dr Willy Mwangi Edwin
C/o Department of Clinical Studies

REF:FVM BAUEC/2018/152

Dear Dr Mwangi,

23/02/2018

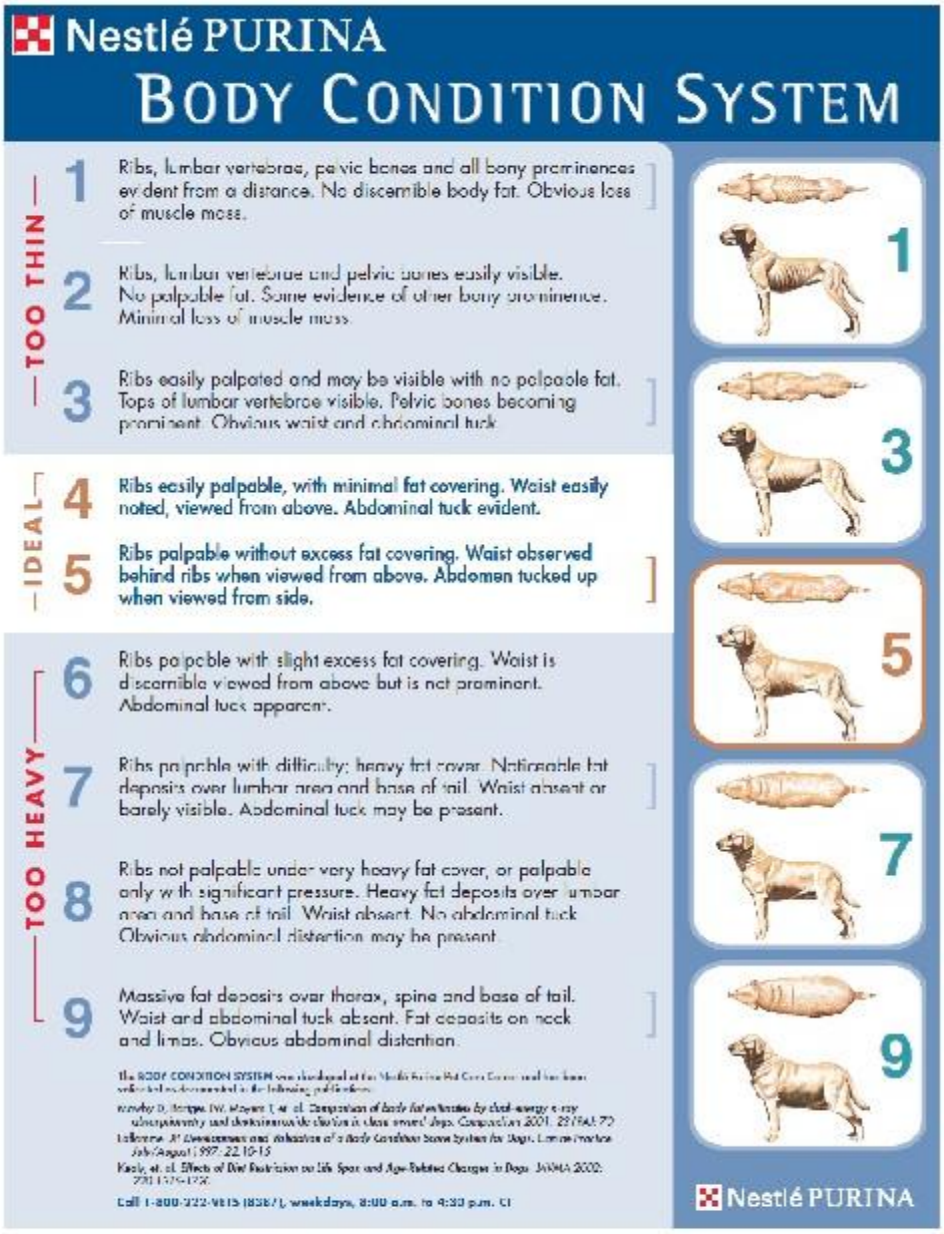
RE: Approval of Proposal by Biosafety, Animal use and Ethics committee
Spatial and molecular epidemiology of Giardia duodenalis in shelter and client-owned dogs in Nairobi County, Kenya
By Dr Willy Mwangi Edwin (Faculty (213390)

We refer to the above proposal that you submitted to our committee for review. We have now reviewed the proposal and have noted that yours is a study involving collection of faecal samples from the two categories of dogs in Nairobi County. We are satisfied that animals will be treated humanely and ethically as per the requirements of this Committee and the necessary biosafety precautions will also be observed.

We hereby approve your study as detailed in your proposal.

Rose O. Ojoo BVM M.Sc Ph.D
Chairman,
Biosafety, Animal Use and Ethics Committee,
Faculty of Veterinary Medicine

APPENDIX 2: BODY CONDITION SCORING SYSTEM



APPENDIX 3: FECAL CONSISTENCY SCORING SYSTEM

APPENDIX 4: QUESTIONNAIRE: THE STANDARDIZED DATA COLLECTION SHEET FOR COLLECTING INFORMATION RELATED TO RISK FACTOR PREDISPOSING DOGS TO GIARDIASIS

Date	Animal Number	Clinic Number
Giardia snap test results	☐ Positive	☐ Negative
Clients Physical address/ Area where dog originated	(Indicate estate/residential area or area of dogs origin)	
GPS co-ordinates for clients Physical address/ Area where dog originated	Latitude	Longitude
Breed	Body weight	Age in months
Gender	☐ Male	☐ Female
Neuter Status	☐ Entire	☐ Neutered
Vaccination	☐ Up to date	Indicate vaccine if

	☐ Vaccinated at least once ☐ Never vaccinated ☐ Don't know	vaccinated ☐ Distemper Hepatitis ☐ Leptospirosis ☐ Parvo enteritis ☐ Rabies Others (Indicate)

		☐
Deworming status	☐ Up to date ☐ Dewormed at least once ☐ Never dewormed ☐ Don't know	☐ Albendazole ☐ Fenbendazole ☐ Praziquantel ☐ Pyrante ☐ Ivermectin
Body condition score *(Tick one box appropriately)*	Kindly check the provided chart and indicate the appropriate score Score_____	

Clinical signs	Vomiting only Duration of signs _____ Diarrhoea only Duration of signs _____ Vomiting and/or Diarrhoea Duration of signs _____ ☐ her significant signs Duration of signs _____

Fecal consistency score *(Tick one box appropriately)*	Kindly check the provided chart for appropriate scoring ☐ Score 1 ☐ Score 2 ☐ Score 3 ☐ Score 4 ☐ Score 5	
Co-infection/Disease (s)	Indicate if there is any other diagnosis apart from *Giardiosis*	
Season *(Tick one box appropriately)*	☐ Dry	☐ Wet

Purpose of keeping	☐ Pet ☐ Security ☐ Laboratory animal ☐ Breeding ☐ Other use
Type of food	

☐ Commercial dog food	☐ Fed often	☐ Fed Occasionally
☐ Homemade food	☐ Fed often	☐ Fed Occasionally
☐ Family food (Food man are eating)	☐ Fed ten	☐ Fed casionally
☐ Others	☐ Fed often	☐ Fed Occasionally

Method Housing *(Tick one box appropriately)*	☐ Group *(2 or more dogs housed in one kennel or in a common compound*)	☐ Individual *(Dog housed in its kennel or alone in a compound)*
Nature of housing	☐ Never kenneled/ roaming ☐ Kennelled during the day and freed at Night ☐ Kennelled throughout	
Ownership	Client ☐	Sheltered ☐