EPIDEMIOLOGY OF CRYPTOSPORIDIOSIS IN DOGS IN LUSAKA DISTRICT, ZAMBIA

BY

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A dissertation submitted to the University of Zambia in partial fulfillment of the requirements of the degree of Master of Science in One Health Analytical Epidemiology

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LUSAKA

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Declaration

I, **LAMSON MUGALA**, do hereby declare that the work presented in this dissertation is my own and has been presented in accordance with the guidelines for MSc dissertation of the University of Zambia. It has not been submitted before for the award of any degree to this or any other University.

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Certificate of Approval

The University of Zambia approves the dissertation submitted by **LAMSON MUGALA**, as fulfilling the partial requirements for the award of the Master of Science Degree in One Health Analytical Epidemiology of the University of Zambia**.**

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Abstract

Cryptosporidium is an intracellular zoonotic protozoan parasite that causes cryptosporidiosis, a diarrhoeal disease of humans and domestic animals. The parasite has been reported in a variety of domestic animals including dogs. Several species of *Cryptosporidium* infecting animals have also been reported in humans highlighting the zoonotic nature of the disease. This study was aimed at determining the prevalence and associated risk factors of *Cryptosporidium* infection in domestic dogs in Lusaka district of Zambia. It was a prospective cross-sectional descriptive study carried out from October, 2015 to May, 2016 in three Veterinary Clinics as well as Kalingalinga and Kabanana residential areas within Lusaka District. A total of 390 dog faecal samples were collected and analyzed at the University of Zambia, School of Veterinary Medicine laboratory and at the parasitology laboratory at the University Teaching Hospital. The modified Ziehl Neelsen and fluorochrome (Auramine) staining techniques were used to identify positive samples. A sample was considered positive if at least one oocyst was identified under the microscope. Proportions were compared using chi-square, fisher's exact test and logistic regression, where appropriate.

Overall, 390 dogs ranging from 2 months to 13 years were examined for the presence of *Cryptosporidium.* Out of these, 280 (71.8%) were of mixed breed while 110 (28.2%) were pure breeds; and 75.6% (295/390) were vaccinated while 24.4% (95/390) were not. Majority of the dogs (62.3%; 243/390) were males compared to only 37.7% (147/390) females. Most of the dogs (89. 2%; 348/390) were fed leftovers while the rest were fed pet food (5.9%; 23/390), sawdust (2.1%; 8/390) or both leftovers and pet food (2.8%; 11/390).

The overall prevalence of *Cryptosporidium* infection in the dogs was 5.9% (23/390). Of the 23 positive dogs, 21 (5.4%) were detected by both Ziehl Neelsen and fluorochrome methods, while the other two were detected by fluorochrome method only, giving a prevalence of 5.9% (23/390) for the latter. The prevalence of *Cryptosporidium* in males was 5.3% (13/243) while that for females was 6.8% (10/147) but the difference was not significant (P= 0.658). There was a statistically significant difference in *Cryptosporidium* infection between mixed breed and pure breed dogs (P=0.012), with prevalence being higher in the mixed breed type. Water source was another variable found to be significantly associated with *Cryptosporidium* infection (P=0.041). Other factors investigated were not associated with *Cryptosporidium* infection.

There was no statistically significant difference in the detection of *Cryptosporidium* using Auramine and Modified Ziehl Neelsen as the results obtained from the two methods were found to be in almost perfect agreement (Kappa=0.95).

The study detected *Cryptosporidium* oocysts in dogs with most of them being asymptomatic. Most of the factors investigated apart from breed and water source were not associated with the *Cryptosporidium* infection. The two compared techniques, namely; Modified Ziehl Neelsen and Auramine can be adopted for routine examination of *Cryptosporidium* oocysts since both showed similar analytical results.

Dedication

This work is dedicated to my wife Lizzy Kasengele Mugala and children Faith Namugala and David Mugala for their unwavering support and encouragement during my studies. My successful completion of this programme was due to their unceasing intercessory prayers and to them I say thank you and may God almighty bless you abundantly. Above all, I acknowledge my Heavenly Father for the health that I have been enjoying.

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List of Abbreviations

AIDS	Acquired Immunodeficiency Syndrome
CBD	Common bile duct
CDC	Centres for Disease Control
CSO	Central Statistics Office
DNA	Deoxyribonucleic acid
DPX	Distyrene plasticizer xylene
EIAs	Enzyme Immunoassays
ELISA	Enzyme - Linked Immunosorbent Assay
FEC	Formol Ether Concentration
FM	Fluorescent Microscope
HIV	Human Immunodeficiency Virus
LDVO	Lusaka District Veterinary Office
LED	Light Emitting Diode
MFNP	Ministry of Finance and National Planning
MMWR	Morbidity and Mortality Weekly Report
MZN	Modified Ziehl Neelsen
N	Sample size
NAIS	National Agriculture and Information Services
NTZ	Nitazoxanide
PCR	Polymerase Chain Reaction
PGE	Prostaglandin E
RAPD	Randomly Amplified Polymorphic DNA

RFLP	Restriction Fragment Length Polymorphism
TNF	Tumour Necrosis Factor
TZ	Tizoxanide
TZglu	Tizoxanide-glucuronide
UK	United Kingdom
UNICEF	United Nations International Children's Emergency Fund
USA	United States of America
WHO	World Health Organization

CHAPTER ONE

INTRODUCTION

1.1 Background

Cryptosporidium is an intracellular zoonotic protozoan parasite that causes cryptosporidiosis, a diarrhoeal disease of humans and domestic animals (Quı´lez *et al*., 2008). Even though the *Cryptosporidium* spp. were first described in 1907 (Tyzzer, 1907), it was not until the 1970s that *Cryptosporidium* was recognized as an important cause of gastrointestinal disease in humans (Leitch and He, 2011). *Cryptosporidium* spp. infect more than 280 species of vertebrate animals and can cause acute or chronic diarrhoea and even death (Fayer, 2010). Over twenty *Cryptosporidium* spp. have been reported but only a few have been found to infect both humans and animals and these include; *Cryptosporidium parvum, Cryptosporidium felis, Cryptosporidium ubiquitum, Cryptosporidium muris, Cryptosporidium meleagridis, Cryptosporidium suis* and *Cryptosporidium canis*. The occurrence of animal *Cryptosporidium* spp. in humans indicates that humans are constantly at risk of contracting cryptosporidiosis from these reservoir hosts. *Cryptosporidium* infections in humans account for up to 6% of all diarrhoea cases in immune competent persons and 24% of persons with both HIV and diarrhoea worldwide (Bialek *et al*., 2002; UNICEF, 2007). *Cryptosporidium* was included in the World Health Organisation's Neglected Diseases Initiative in order to enhance knowledge on the epidemiology and host-parasite interactions especially through molecular techniques (Savioli *et al.,* 2006). The neglected tropical diseases are often indicators of poverty. Those most affected are the poorest populations often living in

remote, rural areas, urban slums, as well as conflict and natural disaster zones where conditions are conducive to the spread of these diseases.

Transmission of *Cryptosporidium* infections is through multiple routes. Infections may be transmitted through person to person, which is particularly important in daycare settings with children; by direct contact with infected animal or via faecal-oral route by ingestion of oocyst contaminated water and food (Khan *et al*., 2004). As low as ten oocysts can cause clinical infections in otherwise healthy persons (DuPont *et al*., 1995). Large outbreaks due to the contamination of water supplies have been documented and in one particular outbreak, contamination of a water-treatment plant in Milwaukee (USA) was estimated to result in infections in 403,000 people (Mac Kenzie *et al*., 1994). *Cryptosporidium* infections continue to be a significant health problem in both developed and developing countries (Harp, 2003), where it is recognized as an important cause of diarrhoea in both immunocompromised and immunocompetent people (Kjos *et al*., 2005). In developing countries, 45% of the children experience an infection before the age of two years (Mor and Tzipori, 2008). Nausea, vomiting, discomfort and low-grade fever are other clinical symptoms often observed (Bouzid *et al.*, 2013).

Studies in Zambia have reported *Cryptosporidium* infection in both humans and animals. In man, it has been shown to range from 6 to 32% (Kelly *et al.*, 1997; Amadi *et al*., 2001; Siwila *et al*., 2007), while in calves it ranges from 6.3 to 42.8% depending on type of farm management system (Geurden *et al*., 2006). Though studies have been done in cattle in Zambia, no studies have been conducted in other domestic animals such as dogs which

habour genotypes that are of public health importance especially in immunocompromised individuals (Meisel *et al*., 1976). This study therefore was aimed at determining the epidemiology of *Cryptosporidium* infections in domestic dogs in order to elucidate the public health importance of the disease in Lusaka.

1.2 Statement of the problem and study justification

Cryptosporidium spp. have been reported in cattle, pigs and humans in Zambia (Geurden *et al*., 2006; Siwila *et al*., 2011; Siwila and Mwape, 2012). However, no study has been done to estimate its prevalence in dogs. The increase in the number of residential areas in Lusaka has led to an increase in dog population as most homes keep dogs for security and pets. The major public health concern of pet ownership is the lack of compliance among some owners to keep the recommended number of dogs, to ensure that they are vaccinated against rabies and to have them treated for helminths (worms) as well as other parasites. It is expected that the fewer the pets one has, the more likely he/she is able to afford basic health care for their animals such as vaccination, prophylaxis for parasites and proper shelter among others, thereby decreasing the chances of disease transmission from these animals including the potentially zoonotic organisms. Among the potentially zoonotic organisms in dogs, enteric pathogens are of particular concern which includes *Cryptosporidium* (Hill *et al*., 2000; Abarca *et al*., 2011). These pathogens if not controlled can cause serious illnesses among children and adults who are immunocompromised. The information gained from this study would be used to educate the dog owners on zoonotic infections that can be acquired from dogs such as *Cryptosporidium* infections and preventive measures against animal-to-human infections.

The findings will further attract molecular studies such as characterization of species in order to determine the prevalence of dog specific *Cryptosporidium* spp. and other pathogens affecting domestic dogs.

1.3 Objectives

1.3.1 General Objective

To determine the epidemiology of *Cryptosporidium* infection in dogs and its associated risk factors in Lusaka district, Zambia.

1.3.2 Specific objectives

i. To determine the prevalence of *Cryptosporidium* spp. infection in dogs in Lusaka district, Zambia.
ii. To identify the risk factors associated with *Cryptosporidium* spp. infection in dogs in Lusaka district.
iii. To compare Modified Ziehl Neelsen (MZN) and Fluorochrome staining techniques for the detection of *Cryptosporidium* spp.

CHAPTER TWO

LITERATURE REVIEW

2.1 Historical perspective

Cryptosporidium, the causative agent of cryptosporidiosis, a diarrhoeal disease, is an apicomplexan protozoan parasite infecting a wide range of vertebrates including man (Xiao and Fayer, 2008). This protozoan parasite was first discovered in a house mouse in 1907 by Ernest E. Tyzzer, an American Biologist (Tyzzer, 1907). It was subsequently found in broad range of domestic animals and birds. In 1955, the parasite was found to be associated with diarrhoea in domestic Turkeys (*Meleagris gallopavo*) (Slavin, 1955), and in 1980, it was implicated in the outbreaks of diarrhoea in calves (Tzipori *et al*., 1980). It was reported in dogs by Tzipori and Campbell (1981) and two years later, its clinical case of cryptosporidiosis was reported (Wilson *et al.,* 1983). In humans, the first case was reported in 1976 (Flanigan and Soave, 1993) and in the 1980's more cases of human cryptosporidiosis emerged due to the advent of HIV/AIDS (Gross *et al*., 1986). In 2004, cryptosporidiosis was added to the WHO's 'Neglected Diseases Initiative' which includes diseases that occur mainly in developing countries and are linked to poverty and lack of access to basic services (Savioli *et al*., 2006). *Cryptosporidium* is reported to be one of the most common parasites of domestic animals (Fayer, 2004; Thompson, 2004). Some of the *Cryptosporidium* spp. such as *C. parvum* and *C. canis* are zoonotic and pose a public health risk (Xiao *et al*., 2004; Caccio *et al*., 2005; Smith *et al*., 2006a). Infection is usually self-limiting, but in immunocompromised individuals, especially in HIV/AIDS patients, the illness is often much more severe affecting the respiratory and biliary tracts, and can be fatal (Shukla *et al*., 2006). Infections are mainly spread through faecal-oral

route, though other routes have been reported. Infectious dose is as low as ten oocysts (DuPont *et al*., 1995). In dogs, *C. canis* was first described in 2001 (Fayer *et al*., 2001) and was later reported in humans (Leoni *et al*., 2007; Xiao *et al*., 2007).

2.2 Taxonomic classification of *Cryptosporidium*

Taxonomy of *Cryptosporidium* is organized as follows: Kingdom, Protista; Subkingdom, Protozoa; Phylum, Apicomplexa; Class, Sporozoasida; Subclass, Coccidia; Order, Eucoccidiorida (Tetley *et al*., 1998; Riordan *et al*., 1999). A lack of consensus still exists in the taxonomy of *Cryptosporidium*. This is mainly due to the fact that members of this protozoan genus in the phylum Apicomplexa were thought to be closely related to the coccidian, but, despite strong morphological similarities to the coccidian throughout the life cycle and the presence of mitochondrion specific genes, it has not been shown that *C. parvum* possesses a mitochondria-like organelle as found in classical Coccidia (Tetley *et al*., 1998; Riordan *et al*., 1999). Moreover, molecular data, based on the 18S rDNA phylogenetic analysis suggest that *Cryptosporidium* may be more closely related to gregarines than to the coccidia, a fact that is also supported by similar life cycle stages in both organisms (Hijjawi *et al*., 2002; Fayer, 2004). The actual relationship has not yet been established.

2.3 Parasite morphology

Cryptosporidium oocyst (figure 2.1) measures 4-5 μm in diameter, is spherical or ovoid in shape, containing four crescentic sporozoites and 1-6 large dark amylopectin-like granules.

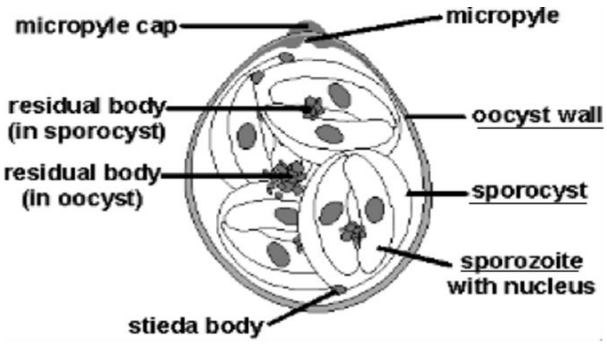


Figure 2.1: *Cryptosporidium* oocyst

(Source: http://www.cdc.gov/parasites/crypto/biology.html)

2.4 Host – specificity

Cryptosporidium infects a wide range of animal species**.** Studies on *Cryptosporidium* isolates obtained from cattle, sheep, pigs, cats, dogs, kangaroos, squirrels and other mammals, have shown that most species are infected with a restricted host-adapted *Cryptosporidium* spp or genotype (Xiao *et al.,* 2004; Xiao and Fayer, 2008). The existence of host-adapted *Cryptosporidium* spp or genotypes indicates that cross transmission of *Cryptosporidium* among different groups of animals is usually limited. However, *C. parvum* has received major attention concerning cross-species transmission (Matsubayashi *et al*., 2004). *Cryptosporidium parvum* was thought to infect all animals. However, it is generally accepted that *C. parvum* infects primarily ruminants and humans. Even in cattle, calves aged less than 2 months are frequently infected with *C.*

parvum (Mendonca *et al*., 2007). In older dairy calves, the majority of infections are caused by *C. bovis* and *Cryptosporidium* deer-like genotype. In adult cattle, *C. andersoni* is the most prevalent parasite. Oocysts of *C. parvum* are not commonly detected in sheep faeces (Castro-Hermida *et al*., 2007) but *C. parvum* infections have been found occasionally in other mammals such as mice and dogs, although companion animals are most often infected with host-specific *Cryptosporidium* spp. (Xiao and Fayer, 2008). Dogs are almost exclusively infected with *C. canis* and cats with *C. felis*. The presence of *Cryptosporidium* in household dogs may cause cryptosporidiosis in humans due to zoonotic transmission of the infection through close contact with the dogs (Tariuwa *et al*., 2007).

2.5 Life cycle

The life cycle of *Cryptosporidium* spp. (figure 2.2) is completed within a single host (monoxenous cycle), and involves both asexual and sexual replication. After ingestion of oocysts by a susceptible host, the first step is excystation, the process by which the oocyst wall opens along a suture to allow the release of four infectious sporozoites (Borowski *et al*., 2008). The sporozoites are not directly in contact with the host cell, and occupy a unique intracellular but extracytoplasmic niche also named parasitophorous sac, typical of *Cryptosporidium* parasites (Valigurova´ *et al*., 2008). There are two types of cycles namely, asexual reproduction where trophozoites nucleus and cytoplasm divide to generate type I and type II meronts and sexual reproduction where merozoites from type II meronts can initiate the sexual phase by differentiating into either a microgamont (male) or a macrogamont (female) which will further divide and differentiate into

microgamete and macrogamete, respectively. Microgamete will fertilize macrogamete and form zygote which will develop into an oocyst which will eventually pass through faeces to the environment, ready to be ingested and start the cycle in another host. Oocysts that sporulate in the respiratory tract are found in nasal secretions and sputum (Mor *et al*., 2010). Two types of oocysts develop during the life cycle of *Cryptosporidium* each containing four haploid sporozoites: thin-walled oocysts, which initiate a new life cycle (auto-invasion), in the same parasitized host by liberating their sporozoites in the gut lumen resulting in chronic infection of the host, and thick-walled oocysts, which are shed in faeces to the environment and are highly resistant to disinfectant and other environmental conditions, so they may be immediately transmitted to the next host without maturation (Campbell *et al*., 1982). The incubation period of cryptosporidiosis varies (seven to 10 days) and depends mainly on the species of the parasite and its host.

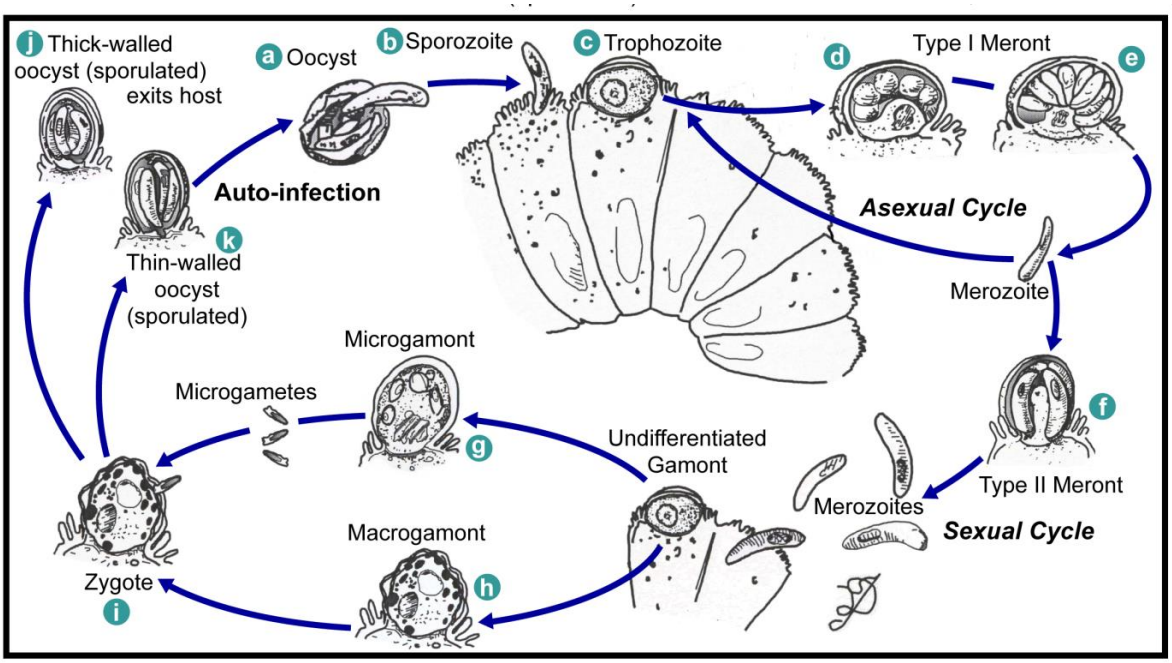


Figure 2.2: Life cycle of *Cryptosporidium*

(Source: https://commons.wikimedia.org/wiki/File:Cryptosporidiosis_01.png)

2.6 Transmission and associated risk factors

2.6.1 Transmission in animals

Animals get infected by *Cryptosporidium* spp. through eating food or drinking water that is contaminated with *Cryptosporidium* oocysts. Feeding pets undercooked or raw meat or letting them get into garbage will predispose them to *Cryptosporidium* infections (Rambozzi *et al*., 2007; Ahmed *et al* 2014). Zelalem and Mekonnen (2012) reported the highest prevalence of gastrointestinal parasites which included *Cryptosporidium* in dogs that were fed raw food (93.7%) followed by dogs that were fed mixed (90.7%) and cooked (37.5%) food items. These findings were supported by Abere *et al*. (2013) who showed that feeding management had a significant influence in the prevalence of *Cryptosporidium* infections. Shelter also plays a very key role in the spread of the *Cryptosporidium* infection in dogs. The prevalence is likely to be more in those dogs that

linger in the streets in search for food in the rubbish and drinking untreated water. Amidou *et al*. (2013**)** found that among the home based dogs, only 5 (41.7%) out of the 12 owned dogs faecal samples were infected whereas the stray dogs had a higher number of *Cryptosporidium* infections i.e. 6 (46%) out of 13 dogs tested.

2.6.1.0 Risk factors in animals

2.6.1.1 Age as a risk factor

Age and sex as risk factors of *Cryptosporidium* infection have been reported in domestic animals (Bajer *et al*., 2012). Studies have linked age of an animal to the prevalence of cryptosporidiosis. Fayer *et al*. (1998) observed that the prevalence of *Cryptosporidium* spp. in pre-weaned calves is usually high. Noordeen *et al*. (2001) found a strong correlation between the age and presence of the protozoan in goat stools, with young animals being more susceptible than adult ones. Other studies reported that puppies were more infected with *Cryptosporidium* spp. compared to adult dogs (Hill *et al*., 2000; Spain *et al*., 2001). Senlik *et al*. (2006) also recorded that, infections were more frequently seen in puppies (0-6 months old) while Overgaauw and Boersema (1998), recorded 21% in adult dogs and 48% in puppies.

2.6.1.2 Sex as a risk factor

Concerning sex as a risk factor for *Cryptosporidium* infections, there are variations in findings from different studies. Some studies have indicated a high prevalence in female dogs compared to male dogs (Abere *et a*l., 2013). To that end, Gbemisola *et al*. (2016) found higher prevalence of *Cryptosporidium* infection in females 20.0% than males (17.68%) in samples examined using ELISA. Another study indicated that female dogs

were more likely to contract *Cryptosporidium* than male dogs (Davoust, 2008). However, Zelalem and Mekonnen (2012) found higher prevalence of 79.2% in male than the 76.8% recorded in female dogs.

2.6.1.2 Breed as a risk factor

Breed has been reported to be associated with prevalence of *Cryptosporidium* infection. Gbemisola *et al*. (2016) found the prevalence to be more in crossbreed of dogs (19.23%) compared to exotic breed. Zelalem and Mekonnen (2012) to the contrary, found prevalence of *Cryptosporidium* in exotic breed dogs to be higher (81.3%) than local breed dogs (76.6%).

2.6.2 Transmission in humans

Humans acquire cryptosporidiosis through multiple transmission routes, including contact with infected animals, person-to-person transmission in households and care settings, consumption of contaminated foods and drinks, consumption of water from private and public supplies, exposure to recreational water in swimming pools or water parks, and travel to endemic countries (Nichols *et al*., 2009). Individuals from developed countries visiting less affluent regions of the world suffer from traveller's diarrhoea (Okhuysen, 2007).

Other significant risk factors for cryptosporidiosis identified especially in developing countries, include age (<2 years), absence of breastfeeding, contact with animals especially among veterinary professionals, living in overcrowded conditions, low birth weight, malnourishment and co-infections that lead to immunosuppression (Putignani and

Menichella, 2010). Cryptosporidiosis is greatly compounded by HIV infection and malnutrition in sub-Saharan Africa. Among HIV-positive children with diarrhoea, prevalence varies between 13.0% as reported in Tanzania (Cegielski *et al*., 1999) and 73.6% in Uganda (Tumwine *et al.*, 2005).

2.6.2.1 Person-to-Person transmission

Cryptosporidium is easily transmitted among children and staff members in nurseries, day care centres and schools (Lee and Greig, 2010). Person-to-person transmission via contaminated hands has been incriminated as the likely route of acquiring infections (Bruce *et al*., 2000). Nosocomial infection can cause secondary cases among roommates and family members (Pandak *et al*., 2006). A case control study in Australia revealed that homosexual men having more than one sexual partner are more likely to have *Cryptosporidium* diarrhoea, indicating that sexual contact represent a risk factor (Hellard *et al*., 2003). Person to person contact through airborne transmission has also been reported from oocysts that sporulate in the respiratory tract and found in nasal secretions and sputum (Mor and Tzipori, 2008). Some studies have speculated that *Cryptosporidium* spp. may be transmitted by Vertical means because the infection was found to be associated with hydrocephalus (Reddy *et al*., 2014) although some investigators have not agreed (Matos *et al*., 2004). However, an experimental study in mice demonstrated vertical transmission of *Cryptosporidium* spp. infection (Kanyari *et al*., 2002).

2.6.2.2 Zoonotic transmission

Domesticated animals serve as reservoirs for *Cryptosporidium* spp. and therefore individuals, who come in contact with animals, either for occupational or recreational reasons, may be at risk of getting infected. Outbreaks of cryptosporidiosis have been reported among veterinarians (Preiser *et al*., 2003; Gait *et al*., 2008), and children visiting farms (Stefanogiannis *et al*., 2001; Hoek *et al*., 2008). Contact with farm animals was identified as a significant risk factor for sporadic cases of human cryptosporidiosis in the UK (Goh *et al*., 2004; Hunter *et al*., 2004). Zoonotic risk factors in case control studies of sporadic cryptosporidiosis cases in England and wales also identified an association between *C. parvum* and touching farm animals or visiting a farm (Hunter and Thompson, 2005). Other domestic animals such as dogs are however also reported as sources of infection as was seen from one study which reported the possible transmission of *C. canis* between a dog and two siblings living in a household in Peru (Xiao *et al*., 2007).

2.6.2.3 Waterborne transmission

Waterborne infectious diseases are a globally emerging public health issue. Various community outbreaks due to contamination of water have highlighted the importance of intestinal protozoa in public health. Among these important pathogens are *Giardia duodenalis*, *Entamoeba histolytica*, *Isospora belli* and *Cryptosporidium* (Karanis *et al*., 2007). Water represents a very important vehicle of infection for the population, and waterborne cryptosporidiosis is a serious public health concern, particularly for populations at risk of severe infection such as pregnant women, children, HIV-positive and organ transplant patients (Smith *et al*., 2006a). Cryptosporidiosis remains the most

frequently reported gastrointestinal illness in outbreaks associated with treated recreational water venues in the UK and the USA (Smith *et al*., 2006b; Yoder and Beach, 2007; Yoder *et al*., 2010; Hlavsa *et al*., 2011). In 1996, Japan experienced a large waterborne outbreak caused by contamination of the town's portable water (Yamamoto *et al*., 2000). During the summer of 2007, (USA) experienced a state wide outbreak of gastrointestinal illness caused by *Cryptosporidium* (CDC, MMWR Report, 2012).

2.6.2.4 Foodborne transmission

Contamination of different types of food with *Cryptosporidium* oocysts has been demonstrated in studies from different regions of the world (Robertson and Chalmers, 2013). A study in Costa Rica investigated the presence of *Cryptosporidium* spp., *Cyclospora* spp., and *Microsporidia* on lettuce, parsley, cilantro, strawberries and blackberries collected from five local markets (Calvo *et al*., 2004). All products except strawberries were found contaminated with *Cryptosporidium*. Another search for parasites in fruits and vegetables was done in Norway from 1999 to 2001 and a total of 29 out of 475 samples analyzed were found to be positive for *Cryptosporidium* oocysts (Robertson and Gjerde, 2001). A few outbreaks have been linked to the consumption of contaminated vegetables. In 2008, *C. parvum* outbreak in Sweden was linked to chanterelle sauce (Insulander *et al*., 2008), while in Finland a salad mixture was the suspected vehicle for a *C. parvum* outbreak (Åberg *et al*., 2015). Contamination of dairy products and fruit juices has also been linked to outbreaks of cryptosporidiosis. The consumption of unpasteurized cow milk has been suggested as the cause of outbreaks in the UK and Australia (Harper *et al*., 2002).

2.6.3.0 Risk factors in humans

2.6.3.1 Age as a risk factor

Cryptosporidiosis is persistent in children especially in developing countries due their naïve immune system, with as many as 45% of children experiencing disease before the age of two years (Valentiner-Branth *et al*., 2003). Cryptosporidiosis has been linked to impaired physical fitness in late childhood (Guerrant et al., 1999) and it has been found to be a significant and independent predictor of childhood death in sub-Saharan Africa (Molbak *et al*., 1993 and Amadi *et al*., 2001).

2.6.3.2 Malnutrition and HIV infections as risk factors

Cryptosporidiosis is greatly compounded by HIV infections and malnutrition in sub-Saharan Africa. Among HIV-positive children with diarrhoea, prevalence varies between 13.0% in Tanzania (Cegielski et al., 1999) and 73.6% in Uganda (Tumwine et al., 2005).

2.6.3.3 Occupation as a risk factor

Veterinary personnel and animal handlers are at risk of contracting infections from animals (zoonotic diseases) because of their frequent contact with a wide variety of species (Langley *et al*., 1995; Mahdi and Ali, 2002).

2.7 Pathogenesis

Once infected, the mean incubation period ranges from seven to 10 days, but it appears to be shorter in the elderly (5–6 days) compared to either children (7 days) or adults (8 days)

(Naumova *et al*., 2003). The virulence factors of *Cryptosporidium* are based on its production of toxins during its life cycle and this has a negative effect on the absorption of chloride ions. It causes villous atrophy which decreases the absorption area. In children with heavier infections, crypt hyperplasia and lymphocyte infiltration are also seen. The parasite also stimulates the abnormal secretion of prostaglandins (PGE), which stimulates the contraction of smooth muscles of the gastrointestinal tract, substance P, responsible for an increase in the vascular permeability of endothelial cells in the intestine, and inflammations, and tumour necrosis factor (TNF), which is also involved in inflammation processes (Tzipori and Ward, 2002). These compounding mechanisms may lead to profuse diarrhoea. Though the disease is self-limiting, it may be chronic in young individuals and those with immunosuppression. This chronicity will lead to undesirable symptoms such as bloody diarrhoea, vomiting, weight loss among others and if not treated adequately it may lead to death (de Oliveira-Silva *et al*., 2007).

2. 8 Clinical features

2. 8. 1 Clinical features in animals

Cryptosporidium infections may either be asymptomatic or symptomatic in animals. In symptomatic infections, the noticeable clinical features include diarrhoea, abdominal pain, nausea or vomiting, mild fever, anorexia, malaise, fatigue and weight loss (Fayer and Ungar, 1986; Casemore, 1990; Hunter and Thompson, 2005). Diarrhoea can be of sudden onset and is generally watery and voluminous; between three and six stools (but sometimes many more) may be passed each day, which are sometimes offensive and may contain mucous. Some of these symptoms are common in both humans and animals. In

dogs, most infections are asymptomatic (Ramírez-Barrios *et al.*, 2004). However, clinical signs have been reported in young puppies. Affected puppies have other concurrent infections like parvovirus enteritis or canine distemper. It is not known whether clinical disease in puppies is solely caused by *Cryptosporidium* infection (Santín and Trout, 2008).

2. 8. 2 Clinical features in humans

Similar to animals, *Cryptosporidium* infections in humans may be asymptomatic and some of the clinical feature noticed in animals may also be noticed in humans. In humans, gastrointestinal cryptosporidiosis presents with moderate to severe watery diarrhea which sometimes contains mucous and rarely contains blood or leukocytes (CDC, 2015). In very severe cases, especially in immunocompromised individuals such as those with HIV/AIDS, diarrhoea may be profuse and cholera-like with malabsorption and hypovolemia (Cabada *et al*., 2015). Other common features include; low grade fever, abdominal pain, dehydration, weight loss and fatigue.

2.8.2.1 Atypical cryptosporidiosis

Gastric involvement during *Cryptosporidium* infections may also lead to very serious complications such as antral narrowing and gastric outlet obstruction which in turn can lead to nausea and vomiting and eventually may cause a severe reduction in nutrient intake (Cersosimo *et al*., 1992). Another unusual complication of *Cryptosporidium* infection is Pneumatosis cystoides intestinalis which is characterized by the presence of

thin-walled, gas-containing cysts in the intestinal wall. Sometimes these cysts can rupture, resulting in a pneumoretroperitoneum and pneumomediastinum (Collins *et al*., 1992).

Respiratory cryptosporidiosis also has been reported involving upper respiratory tract causing nasal discharge, hoarseness, nausea and vomiting and lower respiratory tract causing shortness of breath, cough and fever (Sponseller *et al*., 2014). Cryptosporidiosis was reported to affect oesophagus of a two year old child which was accompanied by vomiting and dysphagia (Kazlow *et al*., 1986). Histological diagnosis also confirmed the association between *Cryptosporidium* infections and appendicitis (Oberhuber *et al*., 1991). Rare signs and symptoms include reactive arthritis, jaundice and ascites due to hepatobiliary and pancreatic involvement respectively (Cabada *et al*., 2015).

Biliary tract infections caused by *Cryptosporidium* spp. have been reported which lead to severe complications such as Cholangitis especially in individuals with HIV/AIDS (Forbes et al., 1993). A study done in Spain among AIDS patients reported that 18.6% of them had *Cryptosporidium* infection of the common bile (Lopez-Velez et al., 1995). *Cryptosporidium* infection induces an inflammatory reaction in the biliary system that results in narrowing and obstruction of the biliary tree. This prevents the bile from draining into common bile duct (CBD) (Tzipori and Widmer, 2008). Pancreatitis is another notable clinical feature involving *Cryptosporidium* infections. Cryptosporidiosis showed that 5 out of 15 autopsies on patients with AIDS had evidence of infection of the

pancreas with histological changes generally mild and limited to hyperplastic squamous metaplasia accompanied with abdominal pain (Godwin, 1991).

2. 9 Epidemiology

2.9.1 Prevalence of *Cryptosporidium* infections in animals

Cryptosporidium lives in soil, food, water and on surfaces that have been contaminated with waste. All these may be the sources of transmission to humans and animals. In humans, *Cryptosporidium* parasite was first recognized in 1976 in a 3 year old child as a causative agent of diarrhoea (Meisel *et al*., 1976). With the advent of HIV pandemic in 1980s, *Cryptosporidium* became widely recognized as an important human pathogen.

Cryptosporidiosis in dogs has been reported in American and European countries, involving both asymptomatic and diarrhoeic dogs. Large-scale surveys of *Cryptosporidium* infection in dogs have been carried out in some countries using different diagnostic methods. The first report of *Cryptosporidium* in dogs was in 1983 in England, UK (Wilson *et al*., 1983). Since then several studies have documented the occurrence, prevalence and risk factors of *Cryptosporidium* in dogs (Bajer *et al*., 2012). *Cryptosporidium* spp. has also been reported from different animals. A study in roe deer (*Capreolus capreolus*) in Galicia (northwest Spain) identified two *Cryptosporidium* spp. (C. bovis and C. ryanae) with a prevalence of 4.2% with more cases reported in juvenile than in adult animals (García-Presedo *et al*., 2013).

A study conducted in São Paulo, Brazil, revealed a prevalence rate of 6.8% for cryptosporidiosis among street dogs and 2.41% in Rio de Janeiro (Huber, 2005). Another study in Brazil reported a prevalence of 10.7% of the 28 dogs tested (Tupler et al., 2012). In Florida, USA, the prevalence of *Cryptosporidium* was 12% among dogs found in an animal shelter. Parasitological study of faecal samples from cats and dogs in Ontario, Canada, showed a high overall positivity rate of 40% (dogs) and 36.6% (cats) (Shukla et al., 2006) while in another comparative study in Costa Rica, 75% of dogs and 67% of cats were found to be infected (Scorza et al., 2011).

In Asian countries, *Cryptosporidium* infections have been reported in both domestic and wild animals ((Paul *et al*., 2014) with India reporting the overall prevalence of 16.2% in all animals studied of which 24.2% was in buffalo calves, 19.1% in piglets, 16.3% in cattle calves, 3.5% in kids and 1.8% in lambs (Maurya *et al*., 2013). Another study in Lahore, Pakistan, reported the prevalence of *C. parvum* in cows and buffalo calves to be 27.2% and 24.0% respectively (Nasir *et al*., 2009). *Cryptosporidium* infections have also been reported in small ruminants with a prevalence of 18.7% being in goats and 21.3% in sheep (Shafiq et al., 2015). A prevalence rate of 9.3% among dogs was reported in Osaka, Japan (Abe *et al*., 2000).

In Africa, prevalence of *Cryptosporidium* infections in domestic animals varies from country to country. In Tanzania and Uganda, prevalence rates of 5.3% and 38%, respectively, of *Cryptosporidium* infections, mostly *C. parvum*, *C. bovis* and *C.*

andersoni were reported in calves (Mtambo *et al*., 1997; Nizeyi et al., 2002). *Cryptosporidium bovis*, *C. ryanae* and *C. andersoni* have also been reported in cattle in Nigeria (Ayinmode and Fagbemi, 2010). Amidou *et al*. (2013) reported a prevalence of 44% and 32% of *Cryptosporidium* infections in dogs and cats respectively in South Africa.

In Zambia, the first study done in animals to estimate the epidemiology and the risk factors of *Cryptosporidium* was in calves and it reported a prevalence of 42.8% in dairy calves, 8.0% in beef calves and 6.3% from traditionally reared calves (Geurden *et al*., 2006). Other prevalence studies on *Cryptosporidium* spp. in Zambia have been conducted in sheep and goats (Goma *et al*., 2007), in intensively managed pigs (Siwila and Mwape, 2012) and in humans (Nchito *et al*., 1998; Amadi *et al*., 2001; Siwila *et al*., 2007). However, no studies have been done to determine the prevalence of *Cryptosporidium* infections in domestic dogs in Zambia.

2.9.2 Prevalence of *Cryptosporidium* infections in humans

Recent outbreaks of cryptosporidiosis in the USA, UK and Ireland have been partially attributed to the presence of *Cryptosporidium* spp. in domesticated animals that pollute the environment (Paudyal *et al*., 2013). A number of studies have investigated the prevalence and epidemiology of cryptosporidiosis in patients with HIV infection. In Europe, cryptosporidiosis seems to affect about 6.6% of HIV-positive individuals (Pedersen *et al*., 1996). Human cryptosporidiosis in Europe and the UK is mostly

waterborne transmission associated with drinking water and swimming pool contact (Karanis *et al*., 2007). However, nosocomial cases were identified at a Hospital in Copenhagen, Denmark where 33% of 60 HIV patients contracted the disease from contaminated ice dispensed from an ice machine in the ward which was contaminated with *Cryptosporidium* oocysts by a psychotic patient (Ravn *et al*., 1991).

In the USA, cases of cryptosporidiosis have been reported among HIV positive children ranging from 3.5% to 8.5% (Pieniazek *et al*., 1999) and the most common species isolated is *C. hominis* followed by *C. parvum* (Widmer *et al*., 1998). A slightly earlier U.S. study in Los Angeles found an overall rate of 3.8% of individuals to be infected with *Cryptosporidium* (Sorvillo *et al*., 1994).

Studies in various countries in South America also have reported the presence of *Cryptosporidium* in HIV infected individuals and the prevalence rates ranges from 4% to 22.8% (Lucca *et al*., 2009) with the more cases occurring in warmer months (de Oliveira-Silva *et al*., 2007).

More cases of cryptosporidiosis were reported in HIV infected individuals in various countries in these continents; however the prevalence has reduced following the introduction of Highly Active Anti-retrol Viral Treatment (HAART) (Inungu *et al*., 2000).

In Asia, *Cryptosporidium* infections have been reported in India in both HIV and non-HIV patients. *Cryptosporidium* infection was reported in HIV and non-HIV patients attending a Hospital with the prevalence of 38.60% and 2.52% respectively, (Maity and

Rahman, 2015). Iran also reported the presence of *Cryptosporidium* in HIV positive individuals ranging from 0.9% to 26.7% (Daryani *et al*., 2009).

Africa has also reported a number of intestinal parasitic infections mainly due to extreme poverty levels coupled with an increase in HIV infections (Assefa *et al*., 2009). Among the intestinal parasitic infections, cryptosporidiosis is the most common, and is of particular concern among HIV-infected individuals. Ethiopia has reported the prevalence of cryptosporidiosis ranging from 11% to 25% (Awole et al., 2003; Getaneh *et al*., 2010) while a prospective cross-sectional study in Nairobi, Kenya revealed *Cryptosporidium* as the most common pathogen with between 17% to 75% cases reported (Mwachari *et al*., 19980). A study in Nigeria reported a prevalence of 52.7% among 100 HIV infected individuals presenting with diarrhoea (Adesiji *et al*., 2007) while a similar study in Zimbabwe reported a prevalence of 9% among HIV infected patients with diarrhoea (Gumbo *et al*., 1999).

In Zambia, *Cryptosporidium* was found to be the most common parasite isolated from 14% of 44 HIV infected Hospitalized children with diarrhoea between the ages 15 months and 5 years (Chintu *et al*., 1995).

2.10 Public health significance

Cryptosporidium is a significant cause of water borne enteric disease throughout the world and represents a challenge to the water industry and a threat to public health. It is

highly resistant to disinfection and even well-operated treatment systems cannot ensure that drinking water will be completely free of *Cryptosporidium* (WHO, 2009). The public health implications of the presence of *Cryptosporidium* spp. in drinking water, and the necessary actions to protect public health, are not as clear or as straightforward as for other pathogens such as *E. coli* because there is no threshold level of *Cryptosporidium* contamination of drinking water that indicates that human illness is likely to occur. Consequently, there are no operational guideline levels for *Cryptosporidium* in drinking water (Fairley *et al*., 1999). Dogs and other domestic animals may shed *Cryptosporidium* oocyst into the environment which may eventually contaminate the water source thereby putting the public at high risk of contracting this diarrhoeal causing protozoan parasite. *Cryptosporidium* infections have negative effects at individual, household and national level. Having more people suffering from cryptosporidiosis will mean low productivity because most of the productive people in all the sectors of the economy are either sick or looking after their sick relatives in either Hospitals or in homes. The cost of treating the sick whether hospitalized or not is a great challenge and most of the funds allocated to other sectors of the economy will be channeled towards diagnosis, treatment and prevention of cryptosporidiosis. Education may be affected since children cannot go to school because of sickness or fear of being stigmatized by their friends. These compounding effects may eventually paralyze the economy. The massive waterborne outbreak of cryptosporidiosis in 1993 in Milwaukee, USA, is a case scenario which caused illness in approximately 403,000 persons and generated substantial healthcare costs and productivity losses (Mac Kenzie *et al*., 1994).

2.11 Diagnosis

2.11.1 Clinical diagnosis

Clinically, cryptosporidiosis can be diagnosed by taking note of signs and symptoms presented by an individual such as diarrhoea, abdominal pain, stomach cramps, dehydration, fever, weight loss, nausea, vomiting and hoarseness among others (CDC, 2015). However, other conditions may present with similar signs and therefore confirmation should be done by laboratory diagnosis.

2.11.2 Laboratory diagnosis

2.11.2.1 Microscopic examination

Faecal samples may be treated differently prior to microscopic examination. Direct smear and formol ether concentration (FEC) methods are done routinely with the latter having an advantage of being sensitive because few parasites in the sample are concentrated thereby increasing the probability of being detected. However, due to the tedious work involved and the long process, many laboratories prefer the direct smear method which in most cases results in misdiagnosis of the intestinal parasites. The smears prepared by either direct or FEC methods can either be examined using wet preparation or dried and stained using MZN and fluorochrome techniques as described below.

2.11.2.1.1 Modified Ziehl Neelsen

This technique uses light microscope and it is the modification of Ziehl –Neelsen stain also known as acid-fast stain, which was first described by two German scientists; the bacteriologist Franz Ziehl (1859–1926) and the pathologist Friedrich Neelsen (1854–

1898). Microscopic examination of *Cryptosporidium* spp. is achieved by staining the stool sample with MZN stain which enables the oocysts to be seen as pinkish red against blue or green background depending on the dye used in the counterstaining step. Unlike other techniques such as ELISA and PCR, the MZN technique has the advantage of being the only technique indicating active infections (Samra, 2013; Omoruyi *et al*., 2014). Furthermore, MZN technique is less costly and therefore it is the mostly used technique in poor resource countries (Samra, 2013). However, one of the notable disadvantages of this method is its low sensitivity which ranges from 37-90% (Tuli *et al*., 2010).

2.11.2.1.2 Fluorochrome staining technique

This technique uses Light Emitting Diode (LED) Fluorescent Microscopes (FM) (figure 2) in resource-limited settings as an alternative to the current light microscopes and mercury vapour FMs (Turnbull *et al*, 2011). The specimen is illuminated with light of a specific wavelength which is absorbed by the fluorophores, causing them to emit light of longer wavelength (of different colour than the absorbed light). With Auramine O staining, *Cryptosporidium* oocysts appear as bright yellow fluorescent when viewed under an excitatory light source.

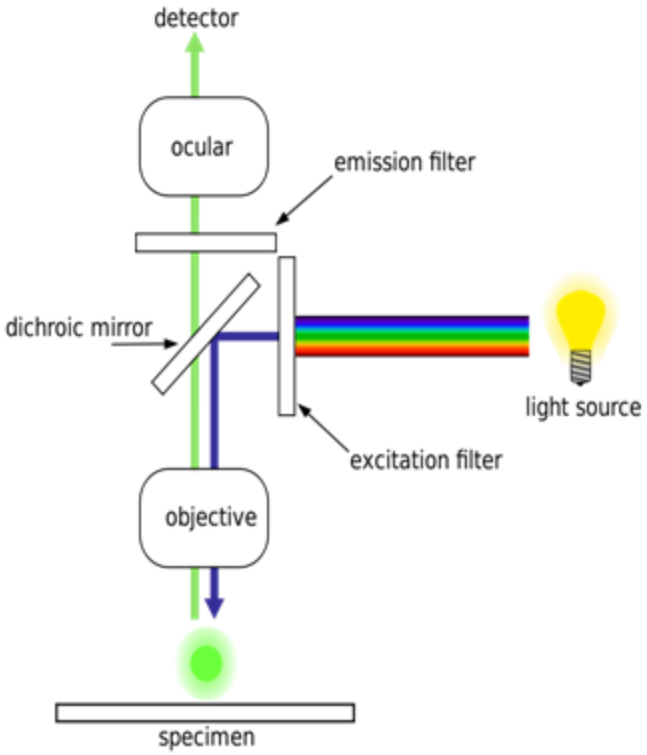


Figure 2.3: LED fluorescence Microscope.
(Source: http://www.fluorescence.com.image/tutoral/dicro).

The sensitivity of this technique is higher than that of light microscope using MZN technique (Omoruyi *et al.*, 2014). However, fluorescent microscopes are very expensive hence this technique is not readily available in most of the routine laboratories in developing countries and it also requires highly skilled manpower otherwise it has the potential to give false positives (Weber *et al*., 1991).

2.11.2.2 Immunoassays

Several immunoassays to detect *Cryptosporidium* spp. in clinical specimens have been devised and the commonly used ones are the enzyme immunoassays (EIAs) which are based on the detection of *Cryptosporidium* antigens in the samples. Enzyme-linked immunosorbent assay (ELISA) is another immunodiagnostic test which has been reported

to be up to 10 times more sensitive than MZN (Katanik *et al*., 2001), making the ELISA method currently the "gold standard" for antigen detection in infected stool samples (Chappell and Okhuysen, 2002).

2. 11. 2. 3 Molecular methods

One of the mostly used molecular methods is the polymerase chain reaction (PCR) which is the most sensitive technique in the detection of *Cryptosporidium* spp. (Kaushik *et al.*, 2008). The technique involves the amplification of the gene of interest using primers that bind to deoxyribonucleic acid (DNA) sequences that are conserved in all the *Cryptosporidium* genotypes (Vanni *et al*., 2012). A number of PCR techniques have been developed to identify *Cryptosporidium* at molecular level using different markers. There are three most common types of markers used today and these are; Restriction Fragment Length Polymorphism (RFLP), Randomly Amplified Polymorphic DNA (RAPD), and isozymes. Of the three marker types, RFLPs have been used the most extensively. RFLP markers have several advantages in comparison with the RAPD and isozyme markers: firstly, they are codominant and unaffected by the environment; secondly, any source of DNA can be used for the analysis; and thirdly, many markers can be mapped in a population that is not stressed by the effects of phenotypic mutations (Garcia *et al*., 2000).

The advantages of PCR method in comparison with other methods is that it has the ability to differentiate between different *Cryptosporidium* genotypes, and can assist in determining the source of cryptosporidial outbreaks (Sulaiman *et al*., 2003). It also has

the advantage of improved sensitivity and specificity, 97-100% and 100% respectively (Omoruyi *et al*., 2014). A study conducted by Morgan *et al*. (1998) compared PCR and MZN in *Cryptosporidium* diagnosis and reported a100% sensitivity for the former and 41.5% for the latter. However, due to high costs, PCR technique has limited applicability (Omoruyi *et al*., 2014) especially in resource poor laboratories.

2.12 Treatment, prevention and control measures

Most infections in immunocompetent animals are self-limiting and recover completely. Treatment, if necessary, is limited to supportive care, particularly fluid replacement (Greene, 2006). Paromomycin, an aminoglycoside, has shown variable efficacy in the treatment of acute cryptosporidiosis in animals in terms of both improvement in clinical signs and decreased shedding of oocysts. Nitazoxanide (NTZ), first-in-class thiazolide is a 5-nitrothiazolyl salicylamide derivative with broad activity against protozoa and helminths. NTZ and its two metabolites, tizoxanide (TZ) and tizoxanide-glucuronide (TZglu) were shown to inhibit the growth of *C. parvum* at concentrations lower than 10 mg/L (Theodos, 1998; Gargala, 2000; Cai *et al*., 2005).

Infection in dogs can be prevented by ensuring that diarrhoeic animals are isolated from other pets and treated as potentially infectious. Disinfecting contaminated areas with bleach where dogs are sleeping is recommended. Faecal samples from animals with suspected cryptosporidiosis should be treated with formalin prior to examination or shipment to an external laboratory to reduce the risk of transmission of infectious oocysts to staff. *Cryptosporidium* oocysts are resistant to all common disinfectants, thus preventing environmental contamination through excellent sanitation is critical.

Thorough cleaning is important to physically remove oocysts from surfaces because disinfection is not reliable for this pathogen. Use of boiling water to scald food and water bowls may also help minimize contamination (Greenberg and Cello, 1996).

Infection control measures such as practicing good hand hygiene, avoiding contact with faeces and preventing faecal contamination of the environment are the primary means of preventing cryptosporidiosis. These measures are especially important for immunocompromised individuals, in whom the consequences of infection, should it occur, can be much more severe (Hunter and Nichols, 2002). The water utility firms should come up with stringent measures to ensure that water supplied to the public is safe. Boreholes dug in residential areas should be in conformity with municipality regulations to avoid contamination from septic tanks (Hunter and Nichols, 2002).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study areas

The study was conducted in three veterinary clinics, Kalingalinga and Kabanana of Lusaka district in Lusaka province of the Republic of Zambia (figure 3.1). Lusaka is the capital city of Zambia and it has a total population of 1,747,152 with about 358,871 households (CSO, 2010).The city covers an estimated area of 360 km^2 and is located at 15º30' latitude south and 28º17' longitude east. The city lies on a plateau 1280m above sea level (MFNP, 2005).

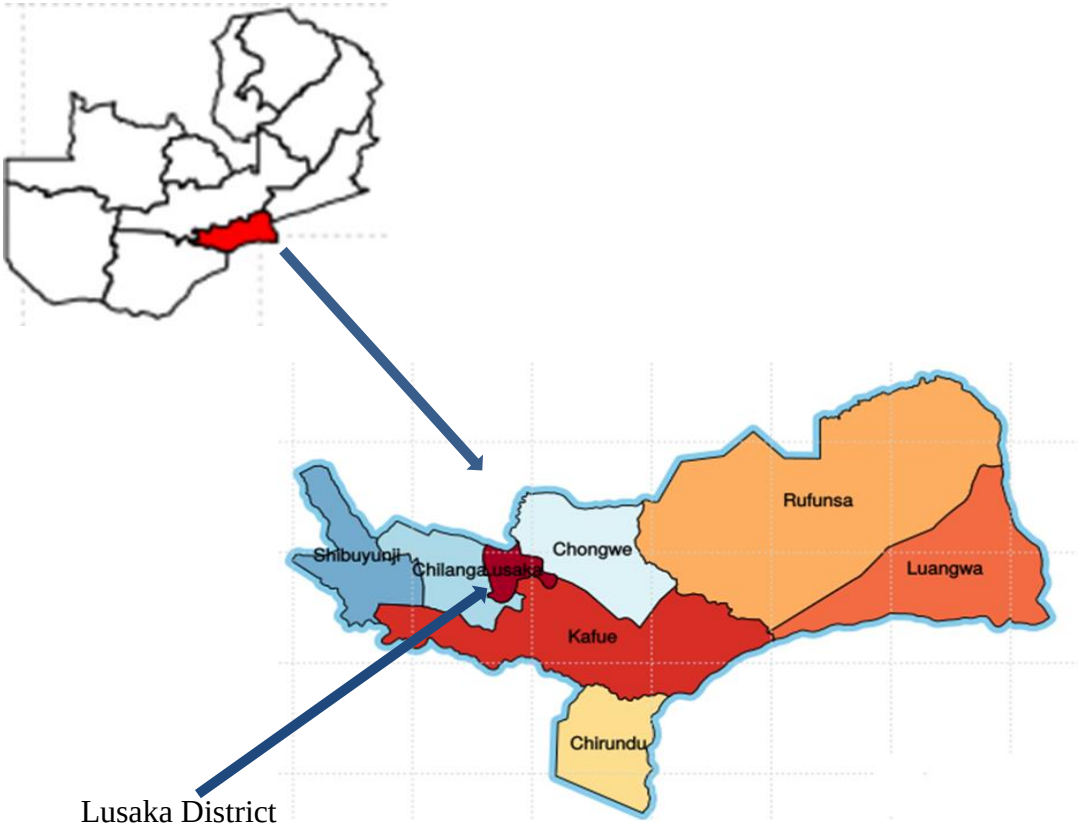


Figure 3.1: Map of Lusaka province showing the location of Lusaka District
Source: https://en.wikipedia.org/wiki/Lusaka_Province#/media/File:Districts of Lusaka Province Zambia.svg

The city has witnessed massive urbanization in recent years. The ever growing population ultimately leads to overcrowding in most of the residential areas.

3.2 Study design

This was a prospective cross-sectional study. All the dogs brought to the selected veterinary clinics were sampled. Furthermore, samples were collected from dogs presented for vaccination in Kalingalinga and Kabanana compounds during a vaccination campaign against rabies. Sampling sites were conveniently selected.

3.3 Sample size calculation

The minimum number of samples in this study was calculated using the following formula as described by Lwanga and Lameshow, (1991).

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

Where:

Z_{α}= 1.96. The value of Z_{α} required for confidence

p= estimate of the proportion of samples containing *Cryptosporidium*

q= 1-p

L= The precision of the estimate (allowable error), equal half of the confidence interval.

50% was used as an estimate of the proportion of samples containing *Cryptosporidium.*

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

n = $\underline{1.96^2 \text{ x } (0.3\text{x } (1\text{- } 0.5))}$

$\underline{(}0.05)^2$

n = 384. Thus, 384 Stool samples were targeted but we sampled 390.

3.4 Inclusion criteria

The study included all the dogs that were taken to the selected veterinary clinics for treatment, vaccination or other procedures.

3.5 Exclusion criteria

Dogs presenting with symptoms of parvovirus enteritis were not included in the study. This was to present possible spread of the parvovirus infection from one dog to another.

3.6 Questionnaire survey

A structured questionnaire was used to capture demographic information of the dogs and hypothesized risk factors such sex, age, diet, breed and management factors among others (Appendix 1). The faecal consistence of each dog (normal, pasty or watery) was noted and recorded in the questionnaire.

3.7 Faecal sampling

The sampling exercise was from October 2015 to April 2016. A single faecal sample was collected *per rectum* from a dog using individual disposable latex glove (Uehlinger *et al*., 2011) as depicted in figure 3.2. The glove was tied off and marked with the dog's identity number and placed in a cool box. The samples were transported to the School of Veterinary Medicine laboratory where they were processed and analyzed. After smears

were prepared, the remaining samples were preserved in 10% formalin solution and in 2ml cryo vials. Samples were preserved by freezing at -20^{o}C.



Figure 3.2: Dog faecal sample collecting technique

3.7 Laboratory analysis of samples

Two methods, namely; Modified Ziehl Neelsen and Auramine were used to diagnose *Cryptosporidium* infection.

3.7.1 Modified Ziehl Neelsen staining procedure

Using the applicator stick, about 0.2g of faecal sample was emulsified onto the slide to make a thick smear which was dried on a slide warmer at 60°C for 5 minutes before fixing it in 70% methanol for 30 seconds as described by Garcia (2001). The fixed smear was put on the staining rack (accommodating 12 slides per rack at a time). The smears were flooded with Kinyoun's carbol fuchsin for 1 minute after which they were rinsed in

distilled water and drained (figure 3.3). The smears were then decolourized in 1% acid-alcohol for 2 minutes after which they were washed again with distilled water and counterstained with malachite green for 2 minutes. Lastly, the smears were washed with distilled water and drained before drying them on slide warmer at 60^0C for about 5 minutes. The stained slides were mounted with the coverslips using Distyrene Plasticizer Xylene (DPX) mounting media and examined under the microscope using oil immersion objectives (x 100 objectives).



Figure 3.3: Staining of smears

3.7.2 Fluorochrome Staining Procedure

Smears made from faecal samples (2 smears, one for MZN staining) were also stained with fluorochrome staining, a method described by Chadwick *et al*. (1958). The smears on well labelled slides were placed on a staining rack and then flooded with Auramine O stain and allowed to stain for 15 minutes. The slides were rinsed with distilled water, followed by decolourization with 0.5% acid alcohol (0.5ml of 70% ethanol in 99.5ml

hydrochloric acid) for 2 minutes. The slides were again rinsed with distilled water and then counterstained with 0.5% potassium permanganate (0.5g potassium permanganate in 100ml distilled water) for 2 minutes. The slides were finally rinsed with distilled water, air-dried and examined within 24 hours using 40x objective of the Fluorescent microscope. To avoid the problem of fading of the Auramine-stain, slides were examined within 24 hours after staining. The *Cryptosporidium* oocysts were seen as shiny yellowish spherical objects against dark background as shown by arrows in figure 3.4.

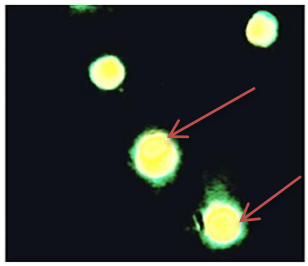


Figure 3.4: Morphological appearance of *Cryptosporidium* on Auramine stain (Source: http://www.cdc.gov/parasites/crypto/biology.html)

3.8 Data analysis

Data was entered in Microsoft Excel® spreadsheet and analyzed using Statistical Package for the Social Sciences (SPSS) version 16. Proportions of positives, with 95% confidence intervals were estimated. The relationships between the presence of *Cryptosporidium* and hypothesized risk factors were investigated using Chi-square or Fisher's exact test in univariate analyses where appropriate. Results were presented in percentages/proportions and the multiple effects of predictor variables were investigated using the logistic regression. A significance level of 5% was used for all tests.

3.9 Ethical consideration

Authority to conduct the study was approved by the University of Zambia and permission to collect dog faecal samples was obtained from Lusaka District Veterinary Office (LDVO), Ministry of Fisheries and Livestock. Informed consent was sought from dog owners during dog recruitment.

CHAPTER FOUR

RESULTS

4.1 Demographic information of dogs

Out of a total of 390 dogs sampled, 243 (62.3%) were males while 147 (37.7%) were females. The age of the dogs ranged from 2 to 156 months (25.04 ± 22.155) and was categorized as tabulated in table 4.1. Majority of the dogs (63.1%) included in the study were adults over 12 months of age. Different breeds of dogs were sampled with most being of mixed breed (71.8%; 280/390) (table 4.1). The few (28.2%; 110/390) pure breed included German Shepard dogs (n = 21)**,** Boerboel (n = 31), Maltese (n = 28), Pitbull terrier (n = 2), Pomeranian (n = 13), Jack Russell (n = 6), Bull dog (n = 1), Boxer (n = 2), Rottweiler (n = 5) and Great Dane (n = 1).

Table 4.1: Demographic information of the dogs (n = 390).

Number of dogs	**Total**	**Total percentages**
	390	100
Sex of dogs		
Males	243	62.3
Females	147	37.7
Age in (months)		
0 - 3	37	9.5
4 - 6	41	10.5
7 - 9	34	8.7
10 - 12	33	8.5
>12	245	62.8
Breed		
Mixed	280	71.8
Pure	110	28.2

The dogs were also categorized into two age groups, that is; those below 12 and those above 12 months which were 145(37. 2%) and 245 (62.8%) respectively.

Out of the 390 samples collected, 206 (52.82%) were from clinics, while 184 were field samples, distributed between Kabanana (116; 29.74%) and Kalingalinga (68; 17.44%) areas (Figure 4.1)

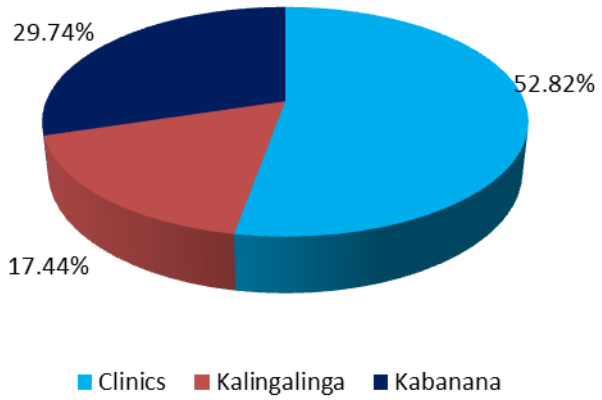


Figure 4.1: Proportions of dogs sampled from each sampling sites

Most dogs (75.6%; 295/390) had received vaccination against rabies while 24.4% (95/390) had not been vaccinated. About 51.3% (200/390) had received deworming tablets according to deworming frequencies tabulated in table 4.3, while 48.7% (190/390) had not been dewormed.

Table 4.2: Dog deworming frequencies

	(n = 390)	
Deworming frequency	**Number of dogs**	**Percentages**
Biannual	59	15.1
Monthly	3	0.8
Not dewormed	190	48.7
Once a year	76	19.5
Quarterly	62	15.9

It was also noted that dogs included in the study were fed different types of diets with most dogs (89.2%; 348/390) being fed on leftovers from the household. Others (5.9 %; 23/390) were fed on a combination of pet food and leftovers while the rest were either fed pet food (2.8%; 11/390) only or on saw dust (2.1%; 8/390). The drinking water source was either municipal (72.8%; 284/390) or borehole (27.2%; 106/390).

4.2 Prevalence of *Cryptosporidium* (Auramine method)

4.2.1 Overall prevalence of *Cryptosporidium*

According to the Auramine method, a total of 23 out of 390 samples (5.9%; 95% CI: 3.9 – 8.7) tested positive for *Cryptosporidium.* The prevalence of *Cryptosporidium* according to sample collection sites was 4.3% (9/206; 95% CI: 2.3 – 8.2) for clinic samples while that for Kalingalinga and Kabanana was 8.8% (6/68; 95% CI: 3.9 - 18.6) and 6.9% (8/116; 95% CI: 3.4 – 13.3), respectively. Although the prevalence of *Cryptosporidium* infection was relatively higher in Kalingalinga, statistically, it was found that sample collection site had no effect on the occurrence of the parasite (p=0.331).

4.2.2 Sex and age related prevalence of *Cryptosporidium*

Of the 147 female dogs sampled, 10 (6.8%) were found to habour *Cryptosporidium* spp oocysts. On the other hand, 5.3% (13/243) male dogs were positive for *Cryptosporidium* oocysts. The difference in the prevalence between males and females was however, not statistically significant (p =0.658)

Lowest prevalence of *Cryptosporidium* was observed in puppies between 0 to 3 months 2.7% (1/37) while highest prevalence was among those aged above 12 months 6.9% (17/245). The age specific prevalence for all the samples analyzed is depicted in Figure 4.2a below and there was no association between the age and the prevalence of *Cryptosporidium* (p=0.905). When the dogs were categorized into two; namely young vs old; that is ≤12 and > 12 months as depicted in figure 4.2b below, it was discovered that the prevalence of *Cryptosporidium* infection was higher in older dogs (6.9%, 17/245) than in younger dogs (4.1%, 6/145) but with no statistical significant (p=0.278).

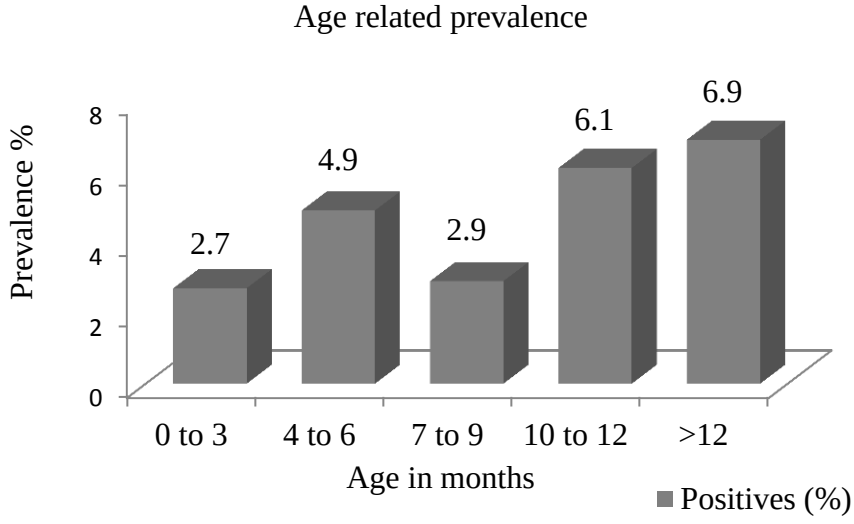


Figure 4.2a: Prevalence of *Cryptosporidium* infection among four different age groups

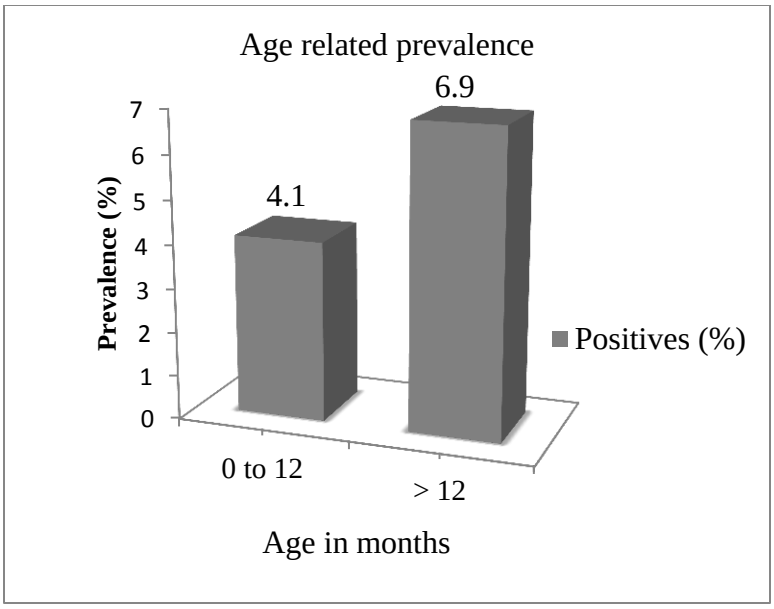


Figure 4.2b: Prevalence of *Cryptosporidium* infection among two different age groups

4.2.3 Breed related prevalence of *Cryptosporidium*

Most of the dogs included in the study were of mixed breed. The prevalence rate of *Cryptosporidium* infection was higher in mixed breed dogs than in pure breeds, though not statistically significant (p = 0.149) (Table 4.3).

Table 4.3: Breed related distribution of *Cryptosporidium* infection in Lusaka

Variable	No. tested	No positive (%)	P value
Breed			
Mixed	280	20 (7.1)	
Pure	110	3 (2.7)	0.149*
Total	**390**	**23 (5.9)**	

*Fisher's Exact Test

4.2.4 Prevalence of *Cryptosporidium* according to faecal consistency

From the 390 samples collected, 22 were diarrhoeic (watery) while the rest (368) were non-diarrhoeic. All the 22 diarrhoeic samples tested negative for *Cryptosporidium* spp. All the 23 (5.9%) positive samples were non diarrhoeic. There was no association between the prevalence of *Cryptosporidium* and diarrhoea (p= 0.631).

4.3 Prevalence of *Cryptosporidium* (Ziehl Neelsen method)

4.3.1 Overall prevalence of *Cryptosporidium*

Oocysts were visualized microscopically (figure 4.3) and identified. A total of 21 samples out of 390 samples (5.4%; 95% CI: 3.5 – 8.1) tested positive for *Cryptosporidium*. The prevalence of *Cryptosporidium* varied from site to site as depicted in Figure 4.4, with the highest and lowest prevalence recorded in Kalingalinga and veterinary clinics respectively. The difference was however, not statistically significant (p=0.158).

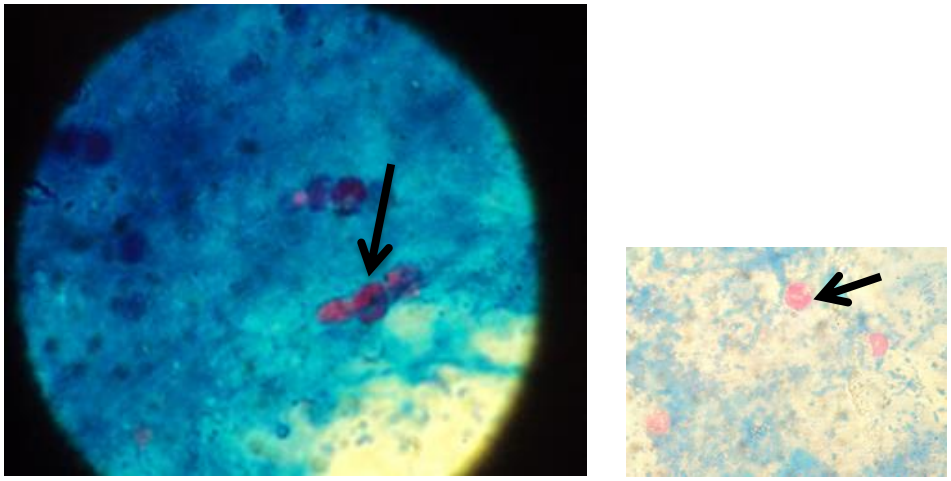


Figure 4.3: Morphological appearance of *Cryptosporidium* on Modified Ziehl Neelsen stained slide using X100 magnification

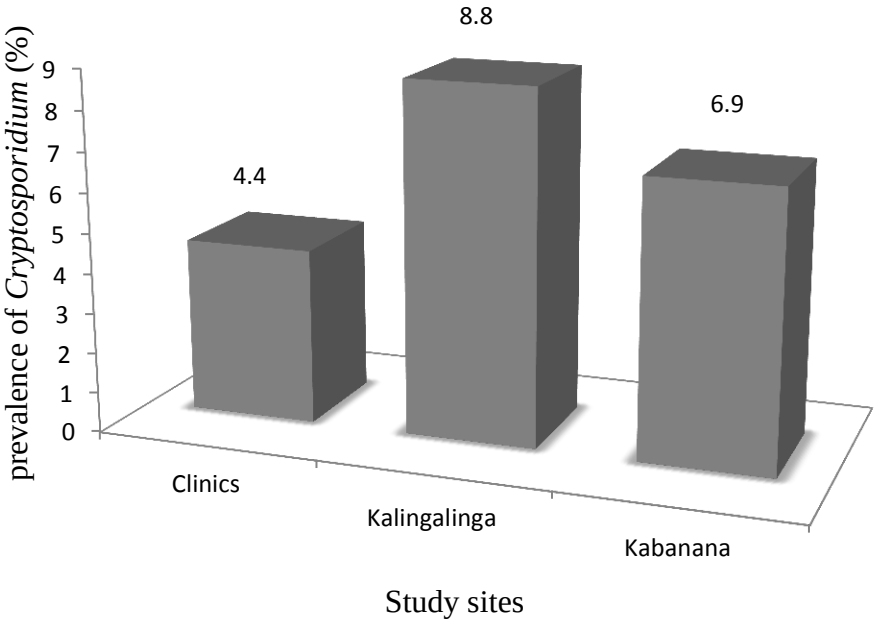


Figure 4.4: Site specific prevalence of *Cryptosporidium* infection

4.3.2 Sex and age related prevalence of *Cryptosporidium*

The MZN method detected 10 (6.8%) positive samples in females, slightly higher than the 4.5% (11/243) detected in male dogs. The prevalence of *Cryptosporidium* between males and females, and among the 4 different age groups was not statistically significant (p=0.360 and p=0.962, respectively). When the dogs were categorized into two age groups; namely those from 0 to 12 months and those above 12 months, it was found that more older dogs tested positive for *Cryptosporidium* (6.1%, 15/245) than the younger dogs (4.1%, 6/145) but with no statistical significant (p=0.491).

4.3.3 Effect of Breed on the prevalence of *Cryptosporidium*

By MZN staining, the distribution of *Cryptosporidium* based on the breed of the dogs is summarized in Table 4.4. It was observed that breed had an effect on the prevalence of *Cryptosporidium* with prevalence being significantly higher in mixed breed type of dogs (p=0.012).

4.2.4 Prevalence of *Cryptosporidium* according to faecal consistency

The prevalence of *Cryptosporidium* based on faecal consistency is shown in Table 4.4 below. Faecal consistency had no influence on the occurrence of *Cryptosporidium* infection in the dogs (P= 0.621).

Table 4.4: Prevalence of *Cryptosporidium* infection according to breed and faecal consistency

Variable	No. tested	Positive (%)	P value*
Breed			
Mixed	280	20(7.1)	
Pure breed	110	1 (0.9)	0.012
Total	390	21 (5.4)	
Faecal consistency			
Diarrhoeic	22	0 (0)	
Non-diarrhoeic	368	21 (5.4)	0.621
Total	**390**	**21 (5.7)**	

*Fisher's Exact test

4.4 Questionnaire survey

A semi structured questionnaire was administered to dog owners to assess dog management practices and other factors. Among the dewormed dogs (5.0%; 10/200) had *Cryptosporidium* while among those which were not dewormed (6.8%; 13/190) had *Cryptosporidium* with no statistical association between the prevalence of *Cryptosporidium* and the deworming status as well as the deworming frequencies (p=0.521 and p=0.572, respectively) as summarized in tables 4.5 below. On living conditions, majority of the dog owners (82.8%; 323/390) reported that their dogs were kept in an enclosed yard while the rest (17.2%; 67/390) were reported to be semi-stray (Table 4.6). Among the enclosed dogs, 5.3% (17/323) were found to be positive for *Cryptosporidium* infection while on the other hand, 9.0% (6/67) of the non-enclosed dogs tested positive for *Cryptosporidium* spp. but with no significant difference (Table 4.6). Other factors investigated are summarized in table 4.6 below and all except water source

(p=0.041) were found not to be significantly associated with the prevalence of *Cryptosporidium* spp. infection.

Dog owners were also asked if their animals had diarrhoea in the previous three weeks before sampling. Only a few dogs (5.6%; 22/390) were reported to have had diarrhoea. Out of the 22 respondents, 14 reported to have had taken the patients for treatment while eight did nothing. All the dogs that were reported to have had diarrhoea tested negative for *Cryptosporidium* spp. There was no association between the prevalence of *Cryptosporidium* and the presence of diarrhoea (p= 0.631).

Table 4.5: Prevalence of *Cryptosporidium* infections based on Dog deworming status and frequencies

	(n = 390)		
Deworming status	**Number of dogs**	**Positives (%)**	**P value**
Dewormed	200	10 (5.0)	
Not dewormed	190	13 (6.8)	
Total	390	23 (5.9)	0.521
Deworming frequency			
biannual	59	5 (8.5)	
monthly	3	0 (0.0)	
Once a year	76	2 (2.6)	
Quarterly	62	3 (4.8)	
Total	200	13 (6.8)	0.572

Table 4.6: Other factors investigated for *Cryptosporidium* infection

Factor	No. examined	No. positive (%)	P value
Enclosed			
No	67	6(9.0)	
Yes	323	17(5.3)	0.254
Number of dogs at home			
1	143	9(6.3)	
2	141	8(5.7)	
3	68	5(7.4)	
>3	38	1(2.6)	0.810
Others animals kept			
No	310	19(6.1)	
Yes	80	4(5.0)	1.000
Water source			
Municipal	284	21(7.4)	
Borehole	106	2(1.9)	0.041
Diet given			
Leftovers	348	22(6.3)	
Petfood	23	1(4.3)	
Leftovers + Pet food	11	0(0.0)	
Sawdust	8	0(0.0)	1.000

During the questionnaire interview, it was noted that most respondents did not know the recommended number of dogs to be kept per household as stipulated by law (The control of dogs act CAP 247 of 1994 of the laws of Zambia) which recommends two dogs per household. Most respondents reported two (33.6%; 131/390) or three dogs (42.6%; 166/390) to be recommended. Some did not know (17.4%; 68/390) while the rest (6.4%;

25/390) reported one or four dogs to be the recommended number. Knowing or not knowing the recommended number of dogs to be kept per household had no effect on the prevalence of *Cryptosporidium* in the dogs (p= 0.575).

4.5 Auramine and Modified Ziehl Neelsen comparative results

Out of 390 samples analyzed, 21 (5.4%) tested positive on both the Auramine and MZN methods while 2 (0.5%) tested positive on Auramine method alone. The prevalence of *Cryptosporidium* using the Auramine test alone was therefore 5.9%. The Kappa's statistical test showed a fair agreement between the two methods used (Table 4.7).

Table 4.7: Level of agreement between Auramine and MZN

Methods	No. tested	Positives (%)	Kappa's statistic
Auramine	390	23 (5.9)	
MZN*	390	21 (5.4)	0.95

* Modified Ziehl Neelsen

CHAPTER FIVE

DISCUSSION

The prevalence of *Cryptosporidium* spp. in domestic dogs in Lusaka district was determined in this study. The overall prevalence of *Cryptosporidium* was 5.9%. Other studies have previously confirmed the presence of *Cryptosporidium* in dogs with variations in prevalence from place to place, which could be due to climatic and seasonality differences and the laboratory diagnostic techniques used. The prevalence in the current study was lower than the 16% reported in Ontario Canada (Shukla *et al.,* 2006) and 75% reported in Costa Rica (Scorza *et al*., 2011). However, low prevalence levels have also been reported previously such as 1.7% reported in Naples, Italy (Bauer and Cirak, 2004). Studies in Africa also have reported the presence of *Cryptosporidium* in dogs with Egypt and South Africa reporting 1.7% (Ahmed *et al*., 2014) and 44% (Amidou *et al*., 2013) respectively.

Prevalence varied from one residential area to another, though not significantly. The prevalence of *Cryptosporidium*, was relatively higher in Kalingalinga (8.8%) compared to Kabanana (6.9%). These areas are both densely populated with most dogs that are kept being semi-stray. The prevalence of *Cryptosporidium* from the dogs sampled from the clinics was much lower (4.4%). The clinic dogs were coming from different areas of Lusaka including the low and high density areas. With no significant difference in the prevalence of the *Cryptosporidium* from the different sources, the results indicate that all dogs are at risk of infection. However, the relatively lower prevalence in the dogs

sampled from the clinics can be explained by the fact that these dogs probably receive better care in terms of veterinary services, food and shelter than those from the field whose majority owners are within the lower income class and cannot afford the veterinary services.

According to age, puppies between 0 to 3 months were found to have the lowest prevalence of *Cryptosporidium* on both Auramine modified Ziehl Neelsen compared to the other older age groups. Even after regrouping the dogs into two age categories; young and old (< 12 versus > 12 months), younger dogs still had lower prevalence. The findings were however in disagreement with those of Hill *et al*. (2000) and Spain *et al*. (2001) who reported that puppies are more likely to be parasitized than adults. Several other studies have reported higher prevalence in young animals other than dogs (Noordeen et al., 2001 and Bajer *et al*., 2012). Though young puppies are said to be more susceptible to various infections including *Cryptosporidium* due to undeveloped immunity unlike older dogs (Ramírez-Barrios *et al*., 2004), the present study to the contrary, did not find an association between *Cryptosporidium* infections and age.

The sex of the dog had no influence on *Cryptosporidium* infection on both univariate and multivariate analysis. However, the prevalence was relatively higher in female dogs on both Auramine (6.8% vs 5.3%) and MZN (6.8% vs 4.5%) techniques than in male dogs. Similar comparative studies in Nigeria indicated that female dogs were more likely to contract intestinal protozoa than male dogs (*Davoust et al*., 2008), even if the difference

was not significant in our study. However, the results conflict with those of Zelalem *et al.* (2012) who found that prevalence of *Cryptosporidium* was higher in male dogs (79.2%) than female (76.8%) dogs. Gbemisola *et al*. (2016) assumed that the higher prevalence of *Cryptosporidium* infection in females than in male dogs could be due to reduced immunity at certain periods in females' physiologic cycle. Since our study did not find any association between prevalence of *Cryptosporidium* and the sex of the dogs, it may imply that both sexes have equal chances of getting infected if they are exposed to infected or contaminated material.

Breed was found to be significantly associated with the prevalence of *Cryptosporidium* infections with more cases reported in mixed breed than in pure breeds. Our findings are consistent with those of Adejimi and Osayomi (2010) who reported a higher prevalence of *Cryptosporidium* infection in mixed breed than in pure breed of dogs. Swai *et al*. (2010) did a similar study in Tanzania and found the prevalence of *Cryptosporidium* to be higher in pure breed than in mixed breed, contrary to our findings. The higher prevalence reported in mixed breed dogs in our study could be attributed to the fact that most of these dogs were semi-stray and therefore were more exposed to contaminated areas/food which could have been contaminated with *Cryptosporidium* oocysts. These areas (Kalingalinga and Kabanana) are densely populated with limited sanitary facilities, which is more likely to lead to environmental contamination by enteric protozoa including *Cryptosporidium* and hence acting as a source of infection. On the other hand, pure breed dogs are more likely to be kept by economically advantaged communities in low density areas as they are expensive to acquire. The low density areas are likely to have better

hygienic environment, clean water, clean food and access to veterinary services among other things. Therefore, dogs kept in these areas are less likely to scavenge and hence less exposure to parasitic and other infections including *Cryptosporidium*.

Prevalence of *Cryptosporidium* infection was high in dogs without diarrhoea but with no statistical significance. This is in agreement with previous reports that indicated that most infections in dogs are asymptomatic (Ramirez *et al*., 2004). Our findings however, disagree with other researchers who reported a significantly higher prevalence of *Cryptosporidium* in dogs with diarrhoea than in those without diarrhoea (Tariuwa *et al*., 2007). High prevalence of *Cryptosporidium* infections reported in non diarrhoeic dogs in the present study implies that dogs may habour the parasite without showing symptoms but can still shed oocysts. In this study, none of the diarrhoeic dogs tested positive for *Cryptosporidium.* The diarrhoea was probably due to other pathogens such as *Giardia* spp, *Ancylostoma caninum* and *Entamoeba histolytica* among others. It has not been shown previously that diarrhoea in dogs (especially puppies) can solely be caused by *Cryptosporidium* infection as dogs that were previously reported to have diarrhoea and were positive for *Cryptosporidium* infections had other concurrent infections (parvovirus enteritis or canine distemper) (Santín and Trout, 2008).

Other factors considered to be associated with *Cryptosporidium* were investigated. The study reported more cases (6.8%) of *Cryptosporidium* in dogs that had not received any dewormer than the 5.0% reported in those that were dewormed but the difference was not

statistically significant (p=0.521). This slight increase in *Cryptosporidium* infections in non-dewormed dogs could be due to the fact that these dogs were not protected from intestinal worms which could have somehow played a role in weakening the immune system of the dogs thereby creating a favourable environment for the survival and replication of *Cryptosporidium*. The frequency among the dewormed was analyzed and no *Cryptosporidium* oocyst was detected in the dogs which were dewormed on monthly basis partly suggesting reinforcement of immunity through continuous deworming. However, this could have happened by chance and statistically there was no difference between dewormed and non-dewormed dogs in the prevalence of *Cryptosporidium* (p= 0.572). On living conditions, it was noted that dogs that were not enclosed (semi stray) had a higher prevalence of *Cryptosporidium* infection (9.0%) compared to those that were enclosed (5.3%) but with no significant difference. This is in line with what was reported by Rambozzi *et al*. (2007) that outdoor cats and dogs were approximately five times more likely to be infected with *Cryptosporidium* spp. than indoor ones. Higher prevalence of *Cryptosporidium* in non-enclosed dogs can be explained by the fact that these dogs roam in the streets where they may scavenge and eat contaminated food or drink contaminated water thereby increasing the risk of getting infected with *Cryptosporidium* spp. Water source for the household as a possible risk factor for *Cryptosporidium* infection was also investigated. Water source was found to be associated with infection (p=0.041) with dogs from households with municipal water having a higher prevalence compared to those from households with boreholes. *Cryptosporidium* is reported to be resistant to chlorination (Korich *et al*., 1990) which is commonly used to treat municipal water locally. Studies have detected *Cryptosporidium*

in well treated water which supports the reports of resistance to chlorination (Rose, 1990). This means that animals (and humans) consuming such water, if contaminated with *Cryptosporidium* oocysts, can get infected. However, infected dogs in this study could have acquired the infection from other sources while roaming or scavenging.

The number of dogs and other types of animals kept per household had no influence on *Cryptosporidium* infection contrary to other studies which indicated that overcrowding of dogs and constant contact with other animals such as cats can contribute to the high prevalence of *Cryptosporidium* infection in dogs (Bugg *et al*., 1999). Further, the study did not find diet as a risk factor unlike Abere *et al*. (2013), who found an association between the prevalence of *Cryptosporidium* and feeding management.

It was also noted that most respondents did not know the recommended number of dogs to be kept per household as stipulated under CAP 247 of 1994 of the laws of Zambia. It should be noted that compliance with this law would assist in controlling dog populations and ensuring that owners only keep the number of dog that they are able to manage and care for. The control of dogs also assists in reducing the number of stray or semi-stray dogs especially in disadvantaged communities and hence preventing spread of potentially infectious pathogens including zoonotic infections.

The present study also compared two methods in the detection of *Cryptosporidium*, namely; Auramine and MZN techniques. The former detected 5.9% cases of *Cryptosporidium* positive dogs compared to 5.4% by the latter. Kappa statistical test showed a fair agreement between the two methods (kappa 0.95). This finding is in agreement with other studies which indicated no qualitative differences in the results obtained with these staining methods (Brook *et al*. 2008; Khurana *et al*., 2012). Another study was done by Rosiléia *et al*. (2006) where 100% and 80% sensitivity was reported for Auramine and MZN, respectively, with no statistically significant difference. The present study however, contradicts Díaz-Lee *et al*. (2011) who compared the sensitivity of these microscopic techniques to detect the parasite in *Cryptosporidium* positive (known positive) faeces of diarrhoeic calves. The authors found a statistically significant difference with Auramine staining being more sensitive than MZN. Though Auramine in other studies has been reported to be more sensitive than MZN, the choice of stain to use would not be based on the sensitivity of the techniques, but on the methodological advantages and disadvantages of its implementation. In countries with poor resource settings like Zambia where Auramine may not be used due to cost of fluorescent microscopes, MZN, which is economically user friendly, can be recommended because it is as good as the Auramine method according to the results obtained in the present study. MZN has other advantages over the Auramine method apart from being inexpensive in that the smears stained by the former method can be preserved permanently unlike smears stained by the Auramine where slides fade with time (Brook *et al*. 2008).

CHAPTER SIX

CONCLUSIONS, RECOMMENDATIONS AND LIMITATIONS

6.1 Conclusions

This is the first cross sectional study conducted in Lusaka district on the detection of *Cryptosporidium* spp. in dogs and it has revealed the following:

1. *Cryptosporidium* spp is prevalent in dogs with an estimated overall prevalence of 5.9% recorded.
2. Breed and water source were the only factors found to be associated with the prevalence of *Cryptosporidium* in dogs.
3. Most of the dogs infected by *Cryptosporidium* were asymptomatic but can still shed oocysts in the environment and act as a possible source of infection for other animals and humans.
4. The Auramine and MZN staining techniques showed similar results.

6.2 Recommendations

Based on the findings from this study, the following recommendations are made:

1. There is need to regularly test all dogs for *Cryptosporidium* and other intestinal protozoa.
2. Molecular studies are recommended to investigate the specific species that are common in dogs in Lusaka.
3. There is need to investigate other diarrhoea causing parasites not only in dogs but also in other domestic animals.

4. There is need to educate dog owners on dog safe keeping practices in order to reduce the intestinal parasitic infections
5. Despite the relatively higher prevalence recorded by the Auramine method, we recommend that MZN be adopted as the standard method for screening for *Cryptosporidium* oocysts because it is economically user friendly and the smears stained can be preserved permanently unlike smears stained by the Auramine method where slides fade with time.

6.3 Limitations

1. The study did not cover all the seasons hence the effect of seasonality on the prevalence of *Cryptosporidium* could not be determined.

2. Since it was a convenient sampling, some categories of dogs were over represented, a scenario that may have an effect on the association between the prevalence of *Cryptosporidium* and the risk factors investigated..

3. Two methods used could not identify the species; hence the most common *Cryptosporidium* species in dogs could not be determined.

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APPENDICES

APPENDIX I

Questionnaire

A survey to determine the epidemiology and risk factors of *Cryptosporidium* infections in dogs in Lusaka district of Zambia

A. DOG AND OWNER INFORMATION

1. Dog ID		2. Date	
3. Name of dog owner			
4. Address of dog owner			

5. Age of dog:	
6. Gender of dog:	
7. Breed	
8. Main diet of dog:	
9. Vaccinated or not (circle appropriate response)	1. Yes 2. No
10. Vaccines received (circle)	1. Antirabies 2. Parvo 3. Vanguard
11. Previous drug treatment diarrhoea in last 2 weeks (circle)	1. Yes 2. No
12. Length owner has been keeping dogs (specify years or months)	

B. DOG MANAGEMENT PRACTICES

1. How many dogs are kept at home? (Circle letter)

A. One

B. Two

C. Three

D. Others specify……...

2. Are your dogs always enclosed in the yard?

A. Yes

B. No

3. Do you know the recommended number of dogs per household according CAP 247 of 1994 of laws of Zambia?

A. Yes

B. No

If yes, what is the number?

A. one

B. two

C. Three

D. Others specify: …….

4. Do you keep other animals at home other than dogs?

A. Yes

B. No

If yes, which ones? Specify……...

7. Do you deworm your dogs?

A. Yes

B. No

If yes, how often?

A. Once a month

B. Every two months

C. Every three months

D. Other (specify)……..

What type of dewormer was used?

Specify……...

9. When did you last deworm your dogs?

A. Last week

B. two weeks ago

C. Three weeks ago

D. Others specify: …….

10. Have you noticed any form of diarrhoea in your dog in the last 3 weeks?

A. Yes

B. No

If yes, what did you do?.....

C. OTHER INFORMATION

What is the source of your household water?

A. Borehole

B. Council water

C. Well

D. Others specify……...

END OF QUESTIONNAIRE INTERVIEW. THANK YOU VERY MUCH