PREVALENCE AND FACTORS ASSOCIATED WITH *CRYPTOSPORIDIUM* INFECTION AMONG ADULT HIV POSITIVE POPULATION IN CONTACT WITH LIVESTOCK IN NAMWALA DISTRICT, ZAMBIA

BY

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A DISSERTATION SUBMITTED TO THE UNIVERSITY OF ZAMBIA IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTERS OF PUBLIC HEALTH- ENVIRONMENTAL HEALTH

UNIVERSITY OF ZAMBIA

2016

DECLARATION

This dissertation is the original work of **NTAZANA N. SINYANGWE**. It has been produced in accordance with the guidelines for MPH dissertation for the University of Zambia. It has not been submitted elsewhere for a degree at this or another University.

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The University of Zambia approves this dissertation of Ntazana N. Sinyangwe in partial fulfilment of the requirements for the award of the degree in Master of Public Health – Environmental Health.

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ABSTRACT

Cryptosporidiosis is a diarrhoeal disease caused by the parasite, *Cryptosporidium* that can live in the intestine of humans and animals and is passed in the stool of an infected person or animal. In immune compromised individuals, *Cryptosporidium* infection can be serious, long-lasting and sometimes fatal. In Zambia, the burden of *Cryptosporidium* infection in the HIV positive population is unknown and factors associated with this infection are unclear. Therefore, this study was aimed at determining the prevalence of *Cryptosporidium* spp. and identifying factors associated with infection among the adult HIV positive population in contact with livestock in Namwala district of Zambia.

Concurrent cross-sectional surveys were conducted in human and animal populations. Consenting 270 adults receiving Anti-Retro Viral treatment were interviewed and requested to provide stool samples. Stool samples were also collected from 174 calves aged six months and below. All samples were analyzed for *Cryptosporidium* infection using the Merifluor® *Cryptosporidium*/*Giardia* immunofluorescence assay. Prevalence in humans and animals were estimated, association between the outcome variable and putative risk factors were evaluated using the chi-square test. Logistic regression was used to examine the multiple effects of predictor variables on the outcome. The overall human prevalence of *Cryptosporidium* infection was 11.1% (30/270). Among the male participants, 15.6% (16) were positive while 8.3% (14) were positive among females. The frequency of *Cryptosporidium* infection was high in the age group category 50-59 years (18.8%). Participants that kept animals had a relatively higher prevalence of *Cryptosporidium* infection (14.2%) compared to those that did not (7.4%) (p= 0.08). Factors identified to be associated with *Cryptosporidium* infection in the adult HIV positive population were sex and marital status. The odds of male participants being infected was 4.2 compared to females. Marital status predicted infection to *Cryptosporidium*, with the divorced group (P=0.01) and the widowed group (p=0.05) being at high risk. In animals, 21% (36/174) were positive for *Cryptosporidium*. Breed (p=0.03) and areas of production, Chitongo and Maala, were associated with *Cryptosporidium* infection in calves (p = 0.03 and p=0.04, respectively).

The study demonstrated that *Cryptosporidium* infection is a problem in the adult HIV positive population in Namwala district. The concentration of this burden among males further suggests a need for targeted sensitization programs aiming to reach such most at risk communities of humans and to reduce exposure and control infection in the animals too. However, given that this was a small study, there is need to undertake further studies on larger samples so as to understand other factors that may be associated with *Cryptosporidium* infection and identify the genotypes prevailing in the population.

DEDICATION

I dedicate this work to my loving husband Daniel Ndambasia and wonderful parents Peter Gilbert Sinyangwe and Lily Ng'ambi Sinyangwe for their constant support, encouragement and belief in me.

ACKNOWLEDGMENTS

My sincere gratitude goes to Capacity Building in Zoonotic Diseases using Integrated Ecosystems Health Approach (CAPAZOMANINTECTO) NORHED project and University of Zambia Staff Development (SDF) Office for the financial and logistical support towards this study; my supervisors Professor J.B. Muma, Dr. J. Siwila-Saasa, and Professor C. Michelo for their continuous advice and input; the technical staff at Namwala District Hospital, Namwala District Veterinary Office, School of Veterinary Medicine and Central Veterinary Research Institute (CVRI) for their help during data collection.

Further, I would like to appreciate my research colleagues, Monde Ngula, Chisoni Mumba, Wiza Mwasinga and Mwinyi Mwinyi for their company and assistance in the field.

Furthermore, I would like to express my heartfelt appreciation to all members of staff from the School of Medicine and School of Veterinary Medicine for their invaluable input.

Last but not the least, I would like to acknowledge my course mates for their support and encouragement during my study at UNZA and the participants in the study.

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LIST OF ABBREVIATIONS

AIDS	: Acquired Immune Deficiency Syndrome
ART	: Anti-Retroviral Treatment
ARVs	: Anti-Retroviral Drugs
CDC	: Centers for Disease Control and Prevention
CVRI	: Central Veterinary Research Institute
HAART	: Highly Active Anti-Retroviral Therapy
HIV	: Human Immunodeficiency Virus
UNAIDS	: United Nations Program on HIV and AIDS
UNICEF	: United Nations Children's Fund
UTH	: University Teaching Hospital
UNZA	: University of Zambia
WHO	: World Health Organization

DEFINITIONS OF KEY TERMS

The following definitions of key terms apply to this specific study.

Adult:	All individuals 18 years and above.
Cryptosporidiosis:	Disease caused by the parasite *Cryptosporidium.*
Cryptosporidium:	Parasite that causes cryptosporidiosis.
***Cryptosporidium* infection:**	Infection caused by the parasite *Cryptosporidium.*
HIV positive population:	Individuals living with HIV currently on anti-retroviral therapy.
Livestock:	Animals raised for the purpose of home use or profit. Examples include cattle, goats, sheep and pigs.
Prevalence:	Proportion of all samples testing positive against the total number of samples tested.
Risk factor:	Any variable or characteristic of an individual that increases the likelihood of the occurrence *Cryptosporidium* infection.
Zoonoses:	Diseases and infections that can naturally be transmitted from animals to humans and vice versa.

CHAPTER ONE

BACKGROUND

1.1 Introduction

Cryptosporidium infection is one of the important causes of diarrhoeal illnesses worldwide. It particularly afflicts young children and immune-compromised patients (Chalmers and Katzer, 2013). Infections result from ingestion of faecal contaminated food or water, direct person to person contact, or zoonotic spread (Hunter and Thompson, 2005, Putignani and Menichella, 2010). The disease is characterized primarily by watery diarrhoea (Anane, 2011, Bouzid et al., 2013) often with other forms of gastro-intestinal distress, such as include nausea, vomiting, low grade fever, myalgia, weakness, malaise, headache and anorexia (Oliveira-Silva et al., 2007, Bouzid et al., 2013). The worldwide spread of human immunodeficiency virus (HIV) in the 1980s brought these organisms to prominent medical attention even though a few sporadic human cases had been reported previously (Anane, 2011, Lima et al., 2011).

In Zambia, the prevalence of HIV/AIDS is currently estimated at 13 % (Central Statistical Office (CSO) [Zambia] et al., 2014) and *Cryptosporidium* infection in HIV/AIDS patients was estimated at 25 - 32% among adults of reproductive age (Holmes et al., 2003). As much as there has been a significant reduction in the prevalence of AIDS and AIDS-related opportunistic infections due to improved antiretroviral regimes, cryptosporidiosis remains among the most common causes of diarrhoea in AIDS patients (Anane, 2011). Though some prevalence studies have been done in HIV/AIDS patients in Zambia (Kelly et al., 1996; Amadi et al., 2001), no such studies have been conducted in other areas, other than Lusaka. This study therefore was aimed describing the epidemiology of *Cryptosporidium* spp. infection among the adult HIV positive population in contact with livestock in Namwala district of Zambia.

1.2 Statement of the problem

In Zambia, the burden of *Cryptosporidium* infection in the general human population is unknown and factors associated with this infection are unclear. Failure to correctly diagnose cryptosporidiosis is consequently fatal especially for the immune compromised HIV sero-positive individuals. Between 2000 and 2013, the University Teaching Hospital (UTH) parasitology laboratory diagnosed 562 cases of cryptosporidiosis in adults and 324 cases in children (unpublished data, Parasitology laboratory, UTH). As for the provinces and districts, the information on the exact burden is limited. This is because studies done in the past mainly focused on children below the age of five (Nchito et al., 1998, Amadi et al., 2001, Siwila et al., 2010) as well as farm workers (Siwila et al., 2007). A similar study done from four townships in Lusaka province reported that 15 of the 16 adult patients diagnosed with cryptosporidiosis were HIV sero-positive (Kelly et al., 1996). This means that the adult HIV sero-positive population based burden for Lusaka district is still unknown.

Furthermore, it is known that *Cryptosporidium parvum* is zoonotic as bovine isolates have been isolated from humans (Monis and Thompson, 2003, Hunter and Thompson, 2005, Siwila, 2006). In Zambia, studies have been carried out which indicate that *Cryptosporidium* species are common in livestock (Geurden et al., 2006, Siwila and Mwape, 2012, Siwila et al., 2013) with prevalence of 33.8% in calves, 11.5% (17/48) in pigs, 5.9% (1/17) in goats, 7.1% in kids, 10.0% (3/30) in ducks and 14.3% (2/14) in chickens (Siwila, 2006, Siwila et al., 2013) being reported. This implies that there is a risk of human exposure from infected livestock.

1.3 Justification

Currently there is some information on *Cryptosporidium* infection in both humans and animals in Lusaka and Kafue districts. In Namwala district, however, no study has been done in humans except for one in cattle (Geurden et al., 2006). With the increase in livestock rearing in Zambia, there was need to determine the prevalence of *Cryptosporidium* infection and the factors associated with it in immune compromised

adult patients who are considered to be at high risk of infection. The risk is even higher in HIV positive population in frequent contact with livestock. In Namwala, a large population of people is constantly in contact with animals as most are livestock farmers (NDHMT), 2014). This predisposes them further to infection from livestock and hence the need to determine the prevalence of *Cryptosporidium* in the animals to confirm its presence in livestock in Namwala district.

1.4 Research Question

What is the prevalence of *Cryptosporidium* infection and what factors are associated with exposure to *Cryptosporidium* among the adult HIV positive population in contact with livestock in Namwala district, Zambia?

1.5 General Objective

To determine the prevalence of *Cryptosporidium* spp. and identify factors that are associated with its infection among the adult HIV positive population in contact with livestock in Namwala district of Zambia.

1.5.1 Specific Objectives

1. To determine the prevalence of *Cryptosporidium* spp. among the adult HIV positive population in contact with livestock.

2. To determine the prevalence of *Cryptosporidium* spp. in cattle.

3. To identify demographic and environmental factors associated with *Cryptosporidium* spp. infection in humans living in contact with livestock.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

Cryptosporidium infection is one of the important causes of diarrhoeal illnesses worldwide. It particularly afflicts young children and immune-compromised patients (Chalmers and Katzer, 2013). The coccidian protozoa was identified as a veterinary pathogen in 1907 (Tyzzer, 1907), and only recognized as a cause of human diarrhoeal disease in 1976 (Meisel et al., 1976) . The worldwide spread of human immunodeficiency virus (HIV) in the 1980s brought these organisms to prominent medical attention even though a few sporadic human cases had been reported previously (Anane, 2011, Lima et al., 2011).

Cryptosporidiosis is characterized primarily by watery diarrhoea (Anane, 2011, Bouzid et al., 2013) often with other forms of gastro-intestinal distress, such as nausea, vomiting, low grade fever, myalgia, weakness, malaise, headache and anorexia (Oliveira-Silva et al., 2007, Bouzid et al., 2013). Infections result from ingestion of faecal contaminated food or water, direct person to person contact, or zoonotic spread (Hunter and Thompson, 2005, Putignani and Menichella, 2010).

Cryptosporidium infection has increasingly become an important cause of diarrhoea in humans. This is particularly so in resource poor settings of developing countries (i.e. Africa, Asia and South America) with high prevalence of HIV/AIDS (Siwila, 2006, Gupta et al., 2008). Patients in these countries usually go undiagnosed for opportunistic infections for long periods hence presenting late in the case of the disease (Gupta et al., 2008). In Zambia, the prevalence of HIV/AIDS is currently estimated at 13 % (Central Statistical Office (CSO) [Zambia] et al., 2014) and *Cryptosporidium* infection in HIV/AIDS patients was estimated at 25 - 32% among adults of reproductive age (Holmes et al., 2003). As much as there has been a significant reduction in the prevalence of AIDS and AIDS-related opportunistic infections due to improved antiretroviral regimes, cryptosporidiosis remains among the most common causes of diarrhoea in AIDS patients (Anane, 2011).

2.2 Taxonomy

Human cryptosporidiosis is an opportunistic infection caused by parasites of the genus *Cryptosporidium.* These parasites are coccidian protozoa, which belong to the Phylum Apicomplexa. Human illness was formerly thought to be caused by a single species. More recently however, molecular studies have now demonstrated that cryptosporidiosis is caused by at least 15 different species. The most common causes of infection in human cases are *Cryptosporidium hominis*, for which humans are the only natural host, and *Cryptosporidium parvum*, which infects bovines as well as humans (White, 2010, Lima et al., 2011, Pearson, 2014). Several studies have shown that there are 23 species of *Cryptosporidium* collectively found in humans and a wide variety of animals (cattle, pigs, sheep, horses, goats, cats, dogs, kangaroos, chickens, turkeys, fish, ferrets, lizards, mice, tortoises, monkeys and deer) (Fayer, 2010, Traversa, 2010, Ren et al., 2012, Elwin et al., 2012). Seven of these species have been found to infect immune compromised individuals such as the HIV- infected persons. These include the predominant human species (i.e. *C .hominis* and *C. parvum*) and others that include *C. meleagridis, C. felis, C. muris, C. canis and C. suis* whose main hosts are turkeys, cats, mice, dogs and pigs, respectively (Iqbal et al., 2012).

2.3 Transmission and environmental factors

Transmission of *Cryptosporidium* occurs through direct and indirect routes. Direct transmission is through the faecal-oral route, which occurs from infected to susceptible hosts. This also includes; animal-to-animal, animal-to-person, person-to-animal, and person-to-person transmissions (Hunter and Thompson, 2005, Cama et al., 2008, Xiao and Feng, 2008). Indirect transmission involves material and environmental contamination with faecal matter coming in contact with susceptible hosts. These materials include water, food and fomites (clothes and footwear). Environmental contamination occurs when faeces, sewage, wastewater or slurry are released and spread in the environment and is often due to overflow following heavy rainfalls (Xiao et al, 2000; Jiang et al, 2005).

Studies have shown that humans can acquire *Cryptosporidium* infections through both direct and indirect transmission routes. For direct routes, person-to-person transmission has been shown to play a major role in the spread of *Cryptosporidium* infection in the paediatric and elderly populations, especially in day care centers and nursing homes (Xiao et al., 2004, Siwila et al., 2010). Zoonotic infections via direct contact with farm animals have also been reported (Siwila, 2006, Lange et al., 2014).

Many outbreaks of cryptosporidiosis due to indirect routes have been reported in several industrialized nations which have been attributable to contaminated food or water (drinking or recreational) (Anane, 2011). Water has been identified as the main route of *Cryptosporidium* transmission especially in areas where the disease is known to be endemic (Xiao et al., 2004). Outbreaks of foodborne transmission have also been reported though not as much as water outbreaks (Smith and Nichols, 2010).

2.4 Demographic/ Host factors

Immunocompromised individuals are at increased risk of getting cryptosporidiosis when compared to healthy individuals. This is because these individuals either have T-cell immune deficiency (primary or acquired) or are HIV infected with CD4 counts lower than 200cells/mm^3 making then very susceptible to cryptosporidiosis (Chalmers and Davies, 2010). Other populations at risk include children, travelers to foreign endemic countries, pregnant women, bisexual partners of infected patients, day-care personnel, users of communal swimming pools and medical personnel attending to infected patients (Meinhardt et al., 1996, Ramirez et al., 2004) .

Illness is often self-limiting in immune competent people but can be persistent and severe in the immune-compromised patients (Chen et al., 2002, Hunter and Nichols, 2002, Nair et al., 2008, White, 2010, Pearson, 2014). The immune compromised patients include those with HIV/AIDS; those on immunosuppressive drugs e.g. cancer and organ transplant patients; and those with affected immune systems due to inherited diseases like Congenital Immunoglobulin A deficiency (Pearson, 2014). HIV burden on its own causes morbidity

and mortality. Opportunistic infections and cryptosporidiosis inclusive, do accelerate progression of HIV to AIDS.

2.5 Prevalence of *Cryptosporidium* infection

Globally, the exact burden of cryptosporidiosis is still uncertain due to inactive surveillance. However, there is some information from different regions worldwide (Putignani and Menichella, 2010). The prevalence and species distribution of *Cryptosporidium* species differ among regions or countries from which studies have been conducted. There is also variation within specific demographic groups (i.e. children, adults and women). This therefore, creates a complex picture of the epidemiology of cryptosporidiosis (Iqbal et al., 2012).

In Africa, a combination of HIV and extreme poverty has been seen to result in widespread intestinal parasitic infections (Assefa et al., 2009). Studies on cryptosporidiosis among HIV- infected individuals have been documented from 12 countries in Africa with prevalence ranging from 3.8% to 73.6% (Iqbal et al., 2012, Wanyiri et al., 2014). The table below shows a summary of the prevalence from different studies in different countries as compiled by Iqbal et al. (Iqbal et al., 2012). The latest addition was a study done in Kenya which reported 34% prevalence (Wanyiri et al., 2014).

Table 1.1: Summary of the prevalence of cryptosporidiosis from the studies done in Africa

Country	Sample size(n)	Age (years)	Prevalence (%)	Diarrhoea (%)
Cameroon	154	23-58	3.8	29.0
Equatorial Guinea	185	-	18.9	-
Ethiopia	52	15 ->45	11.0	51.1
Ethiopia	214	17 – 67	20.1	45.3
Ethiopia	192	15 – 67	25.0	-
Guinea-Bissau	37	26 – 60	25.0	100.0
Kenya	75	-	17.0	100.0

Nigeria	100	9 – 54	52.7	100.0
Nigeria	101	15 -24	16,8	100
South Africa	101	0.5 – 3	24.8	24.0
South Africa	31	0 - >60	12.5	59.1
Tanzania	89	1.1 – 61	8.5	100.0
Tanzania	127	13 – 65	17.0	48.0
Tunisia	75	-	10.7	37.0
Uganda	91	0 – 5	73.6	81.0
Zambia	44	1.25 – 5	14.0	100.0
Zambia	108	0.5 – 2	26.0	100,0
Zimbabwe	82	20 – 59	9.0	100.0

Source: adapted from Iqbal et al., 2012

2.6 Public health and economic importance of *Cryptosporidium spp.*

Cryptosporidium parvum was first noted as a potential zoonosis in the early 1980s. With subsequent studies this was later verified as a serious human problem transmissible between animals and humans (Chalmers and Davies, 2010).

In humans, especially the young, old and immune compromised, the disease causes severe diarrhoea and sickness which can be fatal (Chen et al., 2002). Major water borne outbreaks can occurred as a result of contamination of drinking water by *Cryptosporidium* (Xiao et al., 2004). Furthermore, cryptosporidiosis is now a disease of significant socioeconomic importance worldwide as a result of its recent increasing consideration as an important food borne pathogen. *Cryptosporidium* has three aspects that ensure a high level of environmental contamination and promote the chances of environmental contamination. Firstly, they have a broad range of host susceptibility including man. Their low-infectious dose (single oocyst) (Pereira et al., 2002) enhances the possibility of infection even in healthy immune competent people, who may shed 108-109 oocysts in a single bowel movement and excrete oocysts for up to 50 days after cessation of diarrhoea. Secondly,

they have small and environmental robust transmissive stages (oocysts). Thirdly, normal disinfectants used in the water treatment industry have little action on them (Putignani and Menichella, 2010).

Cryptosporidiosis is a significant disease in livestock, affecting mostly neonates. The economic losses incurred from *Cryptosporidium* infections in livestock and the threat to human health are major concerns. (Ramirez et al., 2004, Siwila, 2006). Prevention and control measures need to be adopted and regulated in the animal environment. The role of the veterinarian in the diagnosis, treatment and counseling concerning cryptosporidiosis is relevant for the management and prevention of the disease in companion and farm animals, as these animals, especially farm animals, have been implicated as the major source of transmission for humans (Ramirez et al., 2004).

2.7 Treatment and prevention

There is no consistent effective therapy proven curative in the absence of effective immune response (Abubakar et al., 2007). Several drugs have been tried in small randomized controlled clinical trials of HIV- infected adults, including nitazoxanide, paromomycin, spiramycin, bovine hyperimmune colostrum and bovine dialyzable leukocyte extract but none of them has shown consistently effective and durable when used alone with or without concomitant antiretroviral therapy (Amadi et al., 2002, Abubakar et al., 2007). Generally anti parasitic drugs have quite a limited activity on *Cryptosporidium*. Nitazoxanide has demonstrated the ability to hasten a cure in immune compromised patients who have a CD4 count of more than 50 percent. However, in chronically ill AIDS patients emphasis is placed on an effective antiretroviral therapy. *Cryptosporidium* induced gastro intestinal injury may interfere with an adequate anti retro viral therapy. It is therefore beneficial to include anti parasitic drugs in the treatment regimen. Paromomycin alone or in combination with a macrolide has demonstrated to be beneficial in this regard (White, 2010).

In the immune competent cryptosporidiosis is self-limiting, however it may present with debilitating and unpleasant effects. The use of rehydration salts to restore lost body electrolytes is highly beneficial. Rehydration therapy in immune compromised individuals is also beneficial (Chalmers and Davies, 2010).

Since treatment options remain limited, the most important interventions are risk intervention and prevention (Chalmers and Davies, 2010, Kelly, 2011). Cryptosporidiosis is highly infectious and the infectious dose is low, therefore high levels of personal hygiene are imperative. Adequate washing of hands with a sanitizing agent after using the toilet, changing diapers or dealing with a person who has *Cryptosporidium* significantly reduces the spread of the disease (Chalmers and Davies, 2010). Suitable hand washing facilities should be provided and used at open farms, food processing facilities, work places, and public convenient rooms (Hunter et al., 2004). People with cryptosporidiosis should be excluded from the work place, school or other institutional settings until 48 hours after the last diarrhoeal episode, particularly food handlers and staff of healthcare facilities (Chalmers and Davies, 2010).

CHAPTER THREE

Materials and Methods

3.1 Study area

The study was done in Namwala district situated in the Southern Province of Zambia (Figure 3.1). The district is located on the banks of the Kafue River, and therefore, the Kafue plains provide suitable grazing land for livestock mainly cattle whose population is estimated at 300,000 herds (Munyeme and Munang'andu, 2011). Namwala is predominantly rural with an extensive human cattle interaction implementing traditional type of cattle rearing. In 2014, the estimated human population of Namwala district was 106,834 (Central Statistical Office (CSO) [Zambia] et al., 2014).

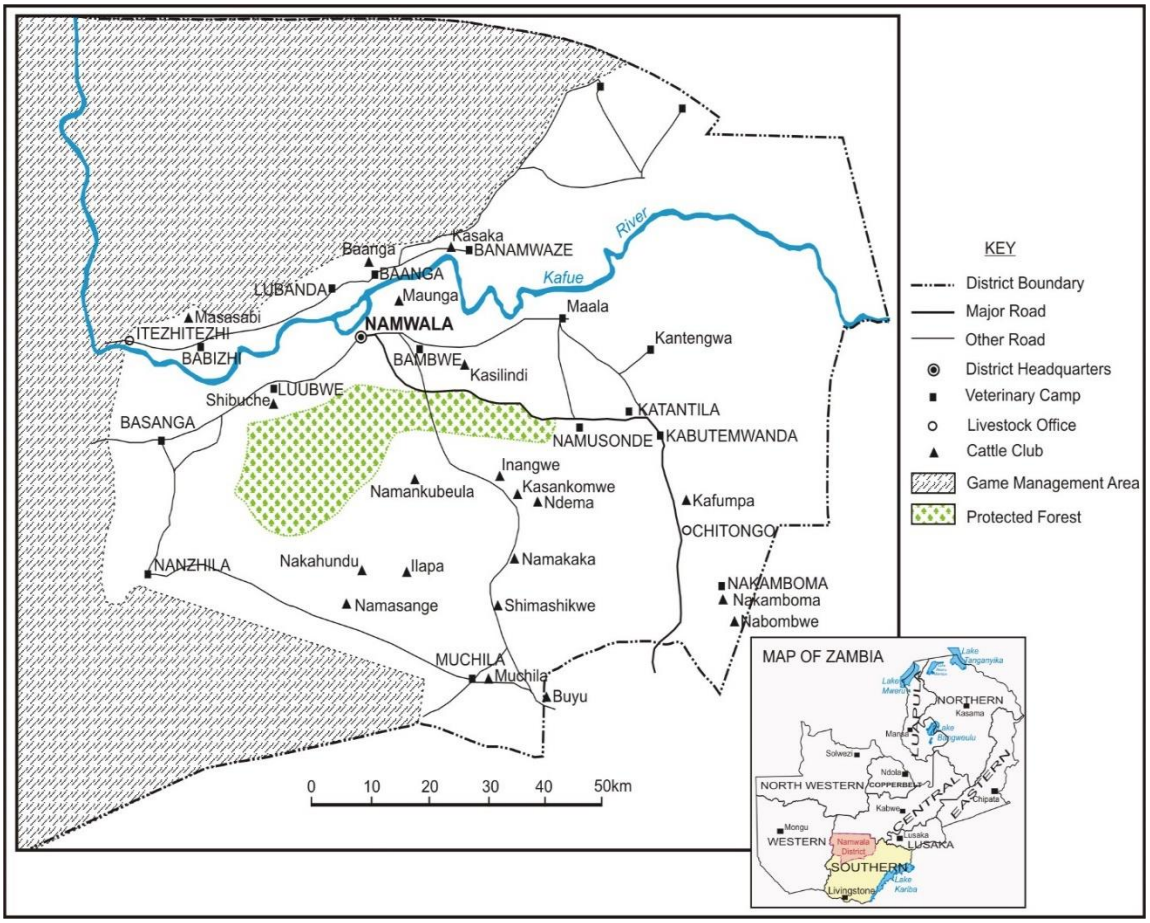

Figure 3.1: Map Showing Namwala District.

The district is predominantly inhabited by the Ila speaking people who are traditional livestock keepers (NDHMT), 2014). The main economic activity is agriculture with livestock farming being one of the major activities in the district. Namwala district has one second level district hospital and 12 rural health centers. It also has one District Veterinary Office with 12 Veterinary Camps. The only industries in Namwala district are the two privately owned abattoirs used for cattle slaughtering whose meat is usually sold to other parts of the country.

3.2 Study design

This was a two tier study conducted in humans and animals. Concurrent cross-sectional surveys were conducted to determine the prevalence and factors associated with *Cryptosporidium* infection. The study was conducted between August 2015 and June 2016.

3.3 Human Survey

3.3.1 Study population

The study population included all adult HIV patients who were receiving Anti Retro-viral Treatment (ART) from Namwala District Hospital, Maala Rural Health Centre, Chitongo Rural Health Centre and Kabulamwanda Rural Health Centre.

3.3.2 Sampling and sample size estimation

The inclusion criteria included all adult (≥ 18 years old) HIV positive individuals who were receiving ART at Namwala District Hospital, Maala, Chitongo and Kabulamwanda Rural Health Centers for over 6 months who consented. All eligible participants who lived in Namwala for less than 6 months, did not consent, pregnant women and persons who were on anti-protozoal treatment were not included in the study. Persons on anti-protozoal treatment were not included in the study because the drugs are used for treatment therapy

for *Cryptosporidium* infection hence high chances of not finding oocysts in these individuals' stool samples. The antiprotozoal drugs are known to reduce oocyst excretion (White et al., 1994)

The sample size was estimated according to the formula: $n = z^2 \times p(1-p)/ \mathcal{E}^2$ (Krejcie and Morgan, 1970):

Where Z = confidence level; P = estimated prevalence; $\mathcal{E}^2$ = error

The following assumptions were considered when estimating the sample size:

i. The estimated *Cryptosporidium* infection in the HIV infected adult population in Zambia = 25-32% (Holmes et al., 2003).
ii. 95% confidence level hence $\alpha = 0.05$
iii. The level of precision or allowable error = 5%.

With the above assumptions, the sample size was then calculated using the given formula as follows: $n = 1.96^2 \times 0.28 \times 0.72/ 0.05^2 = 309.8$. Therefore, the number of persons to be sampled was 310. This sample size was proportionally distributed among the four health centers according to the size of the study population. The list of individuals on ART from each health center was used as sampling frames to select persons to be included in the study. However, this was treated as the target population. Since the total number of individuals on ART was unknown, we aimed to get all who accepted to participate in the study during the period of data collection.

3.3.3 Data collection instruments and techniques

Data collection was done from December 2015 to June 2016. Two hundred and seventy participants were recruited and interviewed using a pre-tested close ended questionnaire (Annex II) in order to collect data on demographic parameters (age, sex, education, employment status, and income), water and sanitation and farm management practices likely to increase the risk for *Cryptosporidium* infection (Table 3.1). The participants were interviewed at the health facilities by the principle investigator and the research assistants

and in instances of language barrier an appointed translator assisted. Prior to this, consent was obtained after explaining the study objectives to the participants.

About 10 – 20g of human faecal sample was collected in stool specimen containers from the participants. Each participant was provided with a container in which they put their stool. The samples were collected from the health facilities and submitted immediately to the research team. Each sample collected was assigned the same identity number as that on the questionnaire. Part of the specimen (stool sample) was transferred into clean cryogenic vials labelled with the identification given on the correspondent specimen containers and stored at-20°C. The other part of the sample was preserved and fixed in 10% formalin until analysis. Samples were later transported in cooler boxes packed with ice packs to the University of Zambia, School of Veterinary Medicine laboratory and the Central Veterinary Research Institute (CVRI) for analysis.

3.3.4 Laboratory sample analysis

The Merifluor® *Cryptosporidium/Giardia* (MERIFLUOR C/G) kit (Meridian Diagnostics Inc., Cincinnati, Ohio, USA) was used for detection of *Cryptosporidium* oocysts in the faecal samples. This assay has sensitivity and specificity of 99% and 100%, respectively (Garcia and Shimizu, 1997) hence it was preferred to be used in this study. Sample analysis was performed according to the manufacturer's instructions as follows:

The specimens stored in 10% formalin were thoroughly mixed before the test procedure. A transfer loop was used to transfer a drop of the faecal material onto a treated slide well. The drop was spread over the entire well. This was done for all the samples using a new transfer loop for each. A new transfer loop was used to transfer a drop of positive control to a separate treated slide well and spread over the entire well. The same was done for the negative control. The slides were then left for 30 minutes to air dry completely at room temperature. A drop of Detection Reagent (anti –*Cryptosporidium* monoclonal antibodies) was placed in each well. This was followed by a drop of Counterstain (Eriochrome black solution) in each well. The reagents were then mixed with an applicator stick and spread over the entire well. Further on, the slides were incubated for 30 minutes in a humidified chamber at room temperature. A wash bottle containing Wash Buffer was then used to

rinse the slides with a gentle stream to remove excess Detection Reagent and Counterstain. Excess buffer was removed by tapping the long age of the slide on a clean paper towel. After that, one drop of Mounting Medium was added to each well and a coverslip was placed. Lastly, each well was examined thoroughly using a fluorescent microscope at 10X magnification for apple green colour as well as the characteristic morphology of *Cryptosporidium* oocysts.

Figure 3.2: Researcher analyzing samples at the University of Zambia, School of Veterinary Medicine.

4.3.5 Measurements

The variables investigated in the study are described in Table 3.1 below. They included the dependent (response) variable and independent (explanatory) variables.

Table 3.1: Dependent and Independent Variables for the Human Participants

Variable	Indicator	Scale of measure
Dependent		
Cryptosporidium infection	Percentage of individuals sampled testing positive over the total number of individuals sampled	Positive/ Negative
Independent		
Demographic factors		
Age	≥ 18 year old	Years
Sex	Sex category	Male/Female
Education	Level of education attained	None Primary Secondary Tertiary
Employment	Gainful employed bringing income	Yes/No
Income	Monthly income from gainful employment or businesses	< 500 501 - 1000 1001- 5000 > 5000
Occupation	What individual does for a living	Livestock rearing, farming, trading, in employment
Environmental factors		
Household water source	Source of water	Shallow well, borehole, river/stream, municipal supply
Animal water source	Source of water	Shallow well, borehole, river/stream, municipal supply
Water treatment	Treatment of household water used for drinking and/or cooking.	Yes/No

Water treatment method	Treatment method used	Boiling or Chlorination
Water contamination by animals	Sharing water source with animals	Yes/ No

3.4 Animal Survey

3.4.1 Study population

This included cattle population in Namwala Central, Maala, Chitongo and Kabulamwanda settlement areas of Namwala district, the same areas where human samples were collected.

3.4.2 Sampling and sample size estimation

Using the formula by Krejcie and Morgan (1970) and assuming an estimated prevalence of traditional cattle at 6.3% (Geurden et al., 2006), 95% confidence level and allowable error 5%, the estimated sample size was 87 herds. We planned to sample 3 calves from each herd bringing the estimated number of calves to be sampled to 261.

3.4.3 Data collection instruments and techniques

A pre-designed data sheet (Annex IIII) was used for bio-data (age, sex and breed) capture of the calves that were sampled after informed consent was given by the owner of the animals. One hundred and seventy-four calves were included in the survey. About 10- 20g of faecal matter was collected from each animal *per rectum* using a gloved hand. Samples collected were stored in 10% formalin and then transported to School of Veterinary Medicine and CVRI for laboratory analysis.

The Merifluor® *Cryptosporidium/Giardia* (MERIFLUOR C/G) kit was used for the detection of *Cryptosporidium* oocysts in the faecal samples according to the manufacturer's instructions as explained above (4.3.6).

3.5 Data Analysis

Before analysis, data from the questionnaires, bio-data sheets and laboratory results was checked for completeness and entered in excel for cleaning. The data was then entered into STATA® statistical package version 13.0 for coding and analysis. The prevalence of cryptosporidiosis among HIV positive patients and cattle was estimated using the survey command in STATA®.

Frequency distributions were reported for all the variables. Chi square test and Fishers exact test (where applicable) were used to evaluate the relationship between the disease outcome and the hypothesized risk factors (predictor variables) in univariate analysis using 0.05 as the level of significance. Further analysis was done using multiple logistic regression to evaluate the multiple effects of these predictor variables on the outcome.

3.5 Ethical Considerations

Ethical clearance was sought from and granted by Excellence in Research Ethics and Science (ERES) Ref. No.2015-June-012 before commencement of the study. Permission to conduct the study was sought from and granted by the Ministry of Health and Ministry of Fisheries and Livestock. The district health officers and veterinary officers were informed of the study and consent was sought from the study participants including the livestock owners before commencement of the study.

Upon arrival at the health center, the study objectives were explained to the medical personnel in-charge and permission was sought in order to conduct the study. The aim of the study was also explained to the research assistants and individuals who were included in the study. Written informed consents were obtained before any interviews and samples were collected from participants who volunteered to be part of the study. Participants were allowed the option of withdrawing from the study when they chose to. Confidentiality of the study results were emphasized to both the research assistants and participants. The respondents were advised that the study was expected to provide information on the presence, magnitude and species of *Cryptosporidium* which would assist the policy

makers in planning future interventions and control measures of the infection. All positive cases were reported back to their health centers for treatment to be given.

Consent was also sought from the animal owners whose calves were included in the study before sampling was done. Trained personnel (Veterinary Assistants) and the Principal Investigator collected fecal samples so as to ensure that the procedure was done with less discomfort to the animals. The animals were stimulated to defecate by rubbing on the dorsal aspect of the rectum using a gloved hand. During sample collection, the animals were not denied access to clean water and grazing.

CHAPTER FOUR

RESULTS

4.1 Human survey

4.1.1 Sample characteristics

Demographic

A total of 270 persons participated in the study and of these, 38% were male and 62% female. Majority of the participants (52%) were aged between 30 and 49 years but the mean age was 35.9 ± 11.9, and median age was 35 (IQR 26 - 43). About 73% of the participants were married, while 10% and 9% were single and widowed, respectively. In terms of education background, most of the participants had attended primary education (63%). Four percent of the participants were in formal employment while the rest (96%) were formally not. Of the employed, only 0.74% had salaries ranging from K1, 000 to K5, 000; this was considered the largest income category. About 2.6% of the employed earned less than K500. Among the 'unemployed', farming was the main form of occupation for the participants (80%) followed by trading (18%). Others which included health workers and teachers accounted for 2%. The description of the demographic characteristics of the study population is given in Table 4.1 below.

Table 4.1: Demographic characteristics for the adult HIV positive population

Variable	n=270 (%)
Sex	
Male	102 (37.8)
Female	168 (62.2)
Age category	
<30	99 (36.7)
30-49	139 (51.5)
50+	32 (11.8)
Marital status	
Single	26 (9.6)
Married	197 (73.0)
Divorced	23 (8.5)
Widowed	24 (8.9)
Education (grouped)	
None	44 (16.3)
Primary	171 (63.3)
Higher than primary	55 (20.4)
Employment	
Yes	10 (3.7)
No	260 (96.3)
Income	
None	258 (95.6)
<500	7 (2.6)
500-1, 000	3 (1.11)
1, 001-5, 000	2 (0.74)
Occupation	
Farming	217 (80.4)
Trading	48 (17.8)
Others	5 (1.8)

Note: Median age = 35 (IQR: 26-43); Median income earned = K750.00 (Range: K150.00 –K4, 000.00)

Environmental:

The main household water source was from boreholes (77%) while streams and rivers accounted for 22% and 9%, respectively. Municipal supply accounting for about 1%. Most of the household water was not treated (Table 4.2). Forty-eight percent of the participants shared their household water sources with animals. Animals' drinking water was mostly from the stream/river (27%). Shallow well and borehole water accounted for 15% and 9%, respectively. Pool/dam water only accounted for about 1% of the animal drinking water source.

Table 4.2: Environmental characteristics for the adult HIV positive population

Variable	**n=270 (%)**			
	Borehole	**Shallow well**	**Stream/River**	**Municipal supply**
Household water source	208 (77.0)	68 (22.2)	24 (8.89)	3 (1.11)
Yes	62 (23.0)	202 (74.8)	246 (91.1)	267 (98.9)
No				
Water treatment method	20 (7.41)	12 (4.44)	10 (3.70)	0
Chlorination	0	2 (0.74)	0	0
Boiling	188 (69.6)	54 (20.0)	14 (5.19)	3 (1.11)
None				

Farm management and practices:

Fifty-five percent of the participants kept livestock with cattle (45.2%) being the most common while sheep was the least kept (3%). Participants' contact with the young animals was mostly with calves (24%); while 11% and 4% had contact with chicks and kids, respectively. Contact with piglets was 2% and that with lambs less than one percent

(0.3%). As for specific types of contact, most of the contact was during feeding. Participants with family members having direct contact with the animals accounted for 53% and the most common type of contact was herding and/ or milking (42%). Most participants practiced extensive/free range type of animal husbandry (Table 4.3).

Table 4.3: Livestock management and practices in Namwala district

Variable	**n=270 (%)**				
	Cattle	**Sheep**	**Goats**	**Pigs**	**Chickens**
Animals kept					
Yes	122 (45.2)	7 (2.6)	34 (12.6)	14 (5.2)	49 (18.2)
No	148 (54.8)	263 (97.4)	236 (87.4)	255 (94.8)	221 (81.8)
Contact with the young animals					
Yes	65 (24.1)	1 (0.3)	11 (4.1)	5 (1.8)	30 (11.1)
No	41 (15.2)	5 (1.8)	19 (7.0)	8 (3.0)	13 (4.8)
Type of contact					
Feeding	31 (11.5)	1 (0.4)	7 (2.6)	2 (0.7)	18 (6.7)
Cleaning	8 (3.0)	0	0	3 (1.1)	2 (0.7)
Others	26 (9.6)	0	5 (1.8)	0	10 (3.7)
Type of husbandry					
Intensive	0	0	0	0	0
Semi-intensive	23 (8.5)	0	14 (5.2)	5 (1.8)	16 (5.9)
Extensive/ Free range	97 (35.9)	6 (2.2)	18 (6.7)	9 (3.3)	30 (11.1)

Eighteen percent (48/270) of the participants or their family members had experienced diarrhoea three weeks before the study was conducted and 6% (16/270) accounted for those individuals that had prior contact with animals. As for animals, 10% (27/270) had experienced diarrhoea in the last three weeks and this was mostly recorded in calves [8% (22/270)]. The detailed description of the burden of disease is given in Table 4.4 below.

Table 4.4: Occurrence of diarrhoea in human participants in Namwala districts

Variable	n=270 (%)
Diarrhoea in humans	
Yes	48 (17.8)
No	222 (82.2)
Prior contact with sick persons/animals	
Yes	16 (5.93)
No	104 (38.5)
Diarrhoea in participants' animals	
Calves	
Yes	22 (8.2)
No	84 (31.1)
Lamb	
Yes	0
No	2 (0.7)
Kids	
Yes	2 (0.7)
No	24 (8.9)
Piglets	
Yes	1 (0.4)
No	10 (3.7)
Chicks	
Yes	1 (0.4)
No	42 (15.6)

4.1.2 Determinants of *Cryptosporidium* infections in humans in Namwala district

The overall prevalence of *Cryptosporidium* infection among the adult HIV positive population was 11.1% (7.9-15.5%) (Table 4.5). This overall prevalence differed by sex (p=0.06) in that it was higher among males (16%) compared to females (8%). Among the different age groups; >30, 30-49 and 50+, the prevalence recorded was 8%, 12% and 19%, respectively. However, the difference among the different age groups was not statistically significant (p = 0.24).

Table 4.5: Proportions of *Cryptosporidium* infection in adult HIV positive sample population

Study area	n=30	*Cryptosporidium* positive	95% CI
Namwala central	9	9.8	5.14 - 17.8
Chitongo	1	6.7	0.86 – 37.1
Kabulamwanda	13	16.2	9.62 – 26.1
Maala	7	8.4	4.04 – 16.8

There were also significant differences (p=0.01) among the different classes of marital status in that participants reported to be divorced had a prevalence of 30%, followed by the widowed and married participants at 17% and 9%, respectively. The lowest burden (4%) was observed among the single participants. Participants who had attended higher education (above primary) recorded more cases of *Cryptosporidium* infection (18%) while those that had never attended school had a prevalence of 14%. About 8% was recorded among participants who had attended primary education. In terms of occupation, participants whose main occupation was trading had the highest prevalence of *Cryptosporidium* infection of about 12% while farming recoded 11%. The differences noted among the different types of occupation were however not significant (P>0.79). These findings are represented in the detailed Table 4.6 below.

Table 4.6: Association of *Cryptosporidium* infection and all demographic characteristics for the human population

Variable	n=270 (%)	*Cryptosporidium* positive	P value
Sex			
Male	102 (37.8)	16 (15.7)	0.06 [c]
Female	168 (62.2)	14 (8.3)	
Age			
<30	99 (36.7)	8 (8.1)	0.24 [c]
30 - 49	139 (51.5)	16 (11.5)	
50+	32 (11.8)	6 (18.8)	
Marital status			
Single	26 (9.6)	1 (3.8)	0.01 [f]
Married	197 (73.0)	18 (9.1)	
Divorced	23 (8.5)	7 (30.4)	
Widow	24 (8.9)	4 (16.7)	
Education			
None	44 (16.3)	6 (13.6)	0.10 [c]
Primary	171 (63.3)	14 (8.2)	
Higher than primary	55 (20.4)	10 (18.2)	
Employment			
Yes	10 (3.70)	1 (10.0)	1 [f]
No	260 (96.3)	29 (11.2)	
Occupation			
Farming	217 (80.4)	24 (11.1)	0.70 [f]
Trading	48 (17.8)	6 (12.5)	
Others	5 (1.8)	0	

Note: [c] denotes analysis by Chi- squared and [f] denotes analysis by Fisher's exact test.

Household water sources did not show any significant results when cross tabulated against *Cryptosporidium* infection. Nevertheless, those using borehole water had 12% prevalence followed by those using shallow wells (10%). The prevalence among those using water from the stream/river was 8% while those connected to municipal supply had none. As for the animal drinking water source, pool/dam had the highest prevalence for animal water source (25%). This was followed by stream/river which accounted for 15% prevalence. Borehole and shallow well both had a prevalence of (12.5%). These differences were however not significant (p=0.31).

Participants that kept animals had a slightly higher prevalence of *Cryptosporidium* infection (14%) compared to those that did not (7%) even though this difference was not statistically significant (p=0.08) at 95% confidence level. As for contact with cattle, males had more contact than females (50.0 vs 42.3%).

The prevalence of *Cryptosporidium* in participants with diarrhoea in the last three month was higher than that in the participants with no diarrhoea (13% vs 11%) though not statistically significant (P=0.74). Participants who had contact with sick persons or animals prior to having diarrhoea was about 19% (3/13). Participants with calves which had a history of diarrhoea were high (29%) compared to those with calves that had not history of diarrhoea (10%) and this was statistically significant (P=0.01).

A multiple logistic regression analysis was carried out for all the underlying variables that had a p value $p \leq 0.1$ at univariate analysis and results are shown in Table 4.7. From the multiple logistic regression, sex and marital status were associated with *Cryptosporidium* infection in the adult HIV positive population. Males were 4.2 times more likely to have *Cryptosporidium* infection than females (p=0.01). Marital status predicted infection to *Cryptosporidium*, with the divorced group and widowed group being at a higher risk of infection compared to other marriage categories (p=0.01 and p=0.05, respectively).

Table 4.7: Results of the logistic regression analysis for the occurrence of *Cryptosporidium* infection in the adult HIV population in Namwala District.

Variable	n=270 (%)	OR (95%CL)	P value
Sex			
Female	168 (62.2)	Ref	
Male	102 (37.8)	4.2 (1.6-11.1)	0.01
Marital status			
Single	26 (9.63)	Ref	
Married	197 (73.0)	2.50 (0.31-19.9)	0.39
Divorced	23 (8.52)	20.3 (2.06-199.5)	0.01
Widowed	24 (8.89)	11.5 (1.05-1.126.2)	0.05

4.3 Animal survey

A total of 174 calves were included in the study and these were equally distributed between male and female calves. The majority of these calves were in age group 9-16 weeks (38%). In the study population, the local breed (*Angoni*) was more represented when compared to the cross breeds (mostly *Angoni* and *Boran*), (59% vs 41%). Majority of the calves sampled were from Maala area. A detailed description of the basic characteristics is given in Table 5.8 below. The faecal consistency of the samples collected was noted and most were firm (87%), 12% were loose or diarrhoeic and about 1% were mucoid.

Table 4.8: Description of basic characteristics of calves sampled in Namwala district (n=174)

Variable	n (%)
Sex	
Male	87 (50)
Female	87 (50)
Age group (weeks)	
0-8	53 (30.5)
9-16	66 (37.9)
17-24	55 (31.6)
Breed	
Local	103 (59.2)
Cross	71 (40.8)
Area of production	
Namwala central	49 (28.2)
Chitongo	29 (16.7)
Kabulamwanda	45 (25.9)
Maala	51 (29.3)

The overall prevalence of *Cryptosporidium* infection in animals was 20.7% (95% CI: 15.3 - 27.4%) and that of different study areas are presented in Table 5.9 below.

Table 4.9: Proportions of *Cryptosporidium* infection in the calves sampled in Namwala district.

Area of production	n= 36	*Cryptosporidium* positive (%)	95% CI
Namwala central	14	28.6	17.5 – 42.9
Chitongo	3	10.3	3.28 – 28.2
Kabulamwanda	7	15.6	7.50 – 29.5
Maala	12	23.5	13.7 – 37.3

This Prevalence was relatively high in male calves (24%) than female calves (17%) although this was not statistically significant (p=0.26). Among the different age group categories, the differences were not statistically significant (P = 0.96). Crossbreed calves had a higher prevalence of C*ryptosporidium* infection compared to the local breed (Table 4:10). In terms of area of production, Namwala central recorded the highest prevalence of *Cryptosporidium* infection (29%) among the production areas even though the differences were not statistically significant. Association of *Cryptosporidium* infection and the calves' background characteristics is given in detail in Table 4.10 below.

Table 4.10: Association of *Cryptosporidium* infection and background characteristics of the calves samples in Namwala district.

Variable	**n (%)**	***Cryptosporidium* positive**	**P value**
Sex			
Male	87 (50)	21 (24.1)	0.26
Female	87 (50)	15 (17.2)	
Age group (weeks)			
0-8	53 (30.5)	11 (20.8)	0.96
9-16	66 (37.9)	13 (19.7)	
17-24	55 (31.6)	12 (21.8)	
Breed			
Local	103 (59.2)	12 (16.5)	0.10
Cross	71 (40.8)	19 (26.8)	
Area of production			
Namwala central	49 (28.2)	14 (28.6)	0.19
Chitongo			
Kabulamwanda	29 (16.7)	8 (10.3)	
Maala	45 (25.9)	7 (15.6)	
	51 (29.3)	12 (23.5)	

On multivariate analysis, breed and area of production emerged as the predictor variables for *Cryptosporidium* infection in cattle of Namwala district. The cross breed calves were 3.8 times (95%CI: 1.13-12.7) more likely to have *Cryptosporidium* infection when compared to the local breeds. Chitongo and Maala areas were less likely to have *Cryptosporidium* infection by 80% and 74%, respectively compared to Namwala central. Table 4.11 below shows the adjusted odds as described here.

Table 4.11: Result for logistic regression analysis for the occurrence of *Cryptosporidium* infection in the calves in Namwala district

Variable	OR (95%CL)	P value
Breed		
Local	Ref	
Cross	3.80 (1.13-12.7)	0.03
Area of production		
Namwala central	Ref	
Chitongo	0.20 (0.05-0.85)	0.03
Kabulamwanda	0.55 (0.19-1.65)	0.26
Maala	0.26 (0.07-0.98)	0.04

CHAPTER FIVE

5.1 DISCUSSION

This study aimed at determining the prevalence of *Cryptosporidium* spp. and identifying factors associated with its infection among the adult HIV positive population in contact with livestock in Namwala district of Zambia. It was established that the overall prevalence of *Cryptosporidium* infection in the adult HIV positive population of Namwala district was 11.1% and the socio-demographic factors; sex and marital status were significantly associated with *Cryptosporidium* infection. However, there was no association observed with the environmental factors as well as the farm management practices.

The presence of *Cryptosporidium* infection in the adult HIV positive population is a public health concern. This is because there is no effective treatment available nor vaccination for *Cryptosporidium* infection for the HIV patients (Snelling et al., 2007, Kelly, 2011). Further, *Cryptosporidium* test positive individuals pose a threat to susceptible persons as well as to the animals in the area (Hunter and Thompson, 2005, Cama et al., 2008, Xiao and Feng, 2008). The prevalence estimated in this study was lower than that reported in Kenya by Wanyiri et al. (2014) which was at 34%. Reasons for this difference can be attributed to use of a polymerase chain reaction (PCR) in their analysis which is more sensitive than the IFAT test used in our study. Further, their study population comprised of untreated HIV positive individuals; bearing in mind that antiretroviral treatment helps to reduce susceptibility to opportunistic infections like *Cryptosporidium* spp. (Anane, 2011).

This study revealed that males were more likely to have *Cryptosporidium* infection compared to their female counterparts. The reason for this difference could be attributed to the fact that males were more in contact with livestock than females in Namwala district. This contact was mostly during daily activities such as herding and milking of the animals. This finding however, does not agree with other scholars' who reported no relationship between gender and *Cryptosporidium* infection (Siwila et al., 2007, Wanyiri et al., 2014).

It is however, highly probable that differences in social responsibilities (looking after livestock in case of males) could account for the observed difference in infection levels between genders in this study.

Significant association was also observed between marital status and *Cryptosporidium* infection, with the divorced and the widowed being at a higher risk compared to the married and singles. These findings do not agree with Wanyiri et al. (2014) who observed the married group to be at risk; although their results were not further disaggregated in various marital categories. The reason why the prevalence was high in the divorced and widowed is not easily discernable but could be attributed to high stress levels giving way to opportunistic infections. In social setups like those pertaining in Namwala, once a woman divorced, they became vulnerable to material poverty since most marriages were customary and most of the financial assets (land and cattle) were usually owned by the household heads, who happen to be males (Mbeza Rural Development Council, 2015).

Age was not associated with *Cryptosporidium* infection in the adult HIV positive individuals in Namwala district and this is in agreement with another scholar (Siwila, 2006) who found no association. However, it was noted that individuals aged 50 years and above had a high prevalence compared to the other age groups (Table 4.6). This finding can be attributed to the fact that the immune system of an individual weakens as they grow older (Makinodan and Kay, 1980) hence they become susceptible to opportunistic infections like *Cryptosporidium* spp.

It was also established that participants who kept animals were two times more likely to be infected than those who did not keep animals. This was, however, not statistically significant but highlights the fact that domestic animals are a risk factor for human infection, as earlier reported (Siwila, 2006, Wanyiri et al., 2014). Participants with young animals that had diarrhoea 3 weeks prior to the study had a high chance of having *Cryptosporidium* infection ($p=0.005$). This shows that contact with cattle, especially calves further increases the risk of exposure to *Cryptosporidium* which is in agreement with observations by other scholars (Ramirez et al., 2004, Siwila, 2006, Wanyiri et al., 2014).

On clinical aspect, the prevalence of *Cryptosporidium* in participants with diarrhoea in the last three month was marginally higher than that in the participants with no diarrhoea, though not statistically significant (P=0.74). These participants were asymptomatic during sample collection. This high prevalence in diarrhoeic participants is in agreement with what has been observed in the study done in Kenya in HIV positive individuals not yet on ART (Wanyiri et al., 2014).

Household water source and sharing of this source with the animals were not associated with the occurrence of *Cryptosporidium* infection in the adult HIV positive population. This is because most of the household water was sourced from boreholes. Water from boreholes in Zambia has low oocyst contamination (Kelly et al., 1997, Siwila et al., 2007).

As for the calves sampled, the overall prevalence was about 3 times more than the 6.3% observed in traditional cattle of southern province (Geurden et al. in 2006). This increase could have been as a result of increase in cattle population (Hamnes et al., 2006) and crossbreeding with exotic breeds in order to improve the quality of the animals. Because of the introduction of exotic breeds, the crossbreed born calves are more susceptible to infection and this association was observed in this study in which crossbreed calves were 3.8 times more likely to have *Cryptosporidium* infection compared to calves from the local cattle breeds. The high prevalence of *Cryptosporidium* spp. infection found in the calves sampled is a public concern. This is because more oocysts could be shed through faecal matter hence increasing the risk of infection to other calves and human beings.

Calves from Chitongo and Maala areas had higher *Cryptosporidium* infection rates compared to those from other areas and the difference was significant. The reason for these areas being at risk of infection in the cattle is probably due to the large populations of cattle kept compared to other areas of production (unpublished data, Namwala District Veterinary Office, 2015). These large populations are at higher risk of having *Cryptosporidium* infection because more oocysts are likely to be shed and be readily available for infecting other susceptible calves (Hamnes et al., 2006). Further, animals in these areas spend most of the time in the plains of the Kafue River and therefore the shed oocysts become readily available for the uninfected susceptible calves.

Cryptosporidium infection was found in the adult HIV positive population in contact with livestock and also in the cattle population. However, an association could not be established from this study because molecular analysis was not done to confirm whether it was the same species in both populations; and the design used could not bring out this association. Furthermore, the source of infection for the human population remains unknown as no genotyping was done.

5.2 Conclusion

Cryptosporidium spp. is prevalent among the adult HIV positive population in contact with livestock in Namwala district. Infections are driven by socio-demographic factors (sex and marital status) and livestock production activities. *Cryptosporidium* spp. is still prevalent in cattle in Namwala district which poses a risk to human.

Therefore, with the knowledge of its prevalence in cattle, this information will be used for public sensitization and health education on the importance of domestic animals as reservoirs/sources of infection in order to reduce the risk of spread from animals to humans and vice-versa and also to control the infection in the animals.

5.3 Limitations

This study however did not go without limitations. The results of the study had limited external validity because a non-probabilistic sampling approach was employed in recruiting participants. Further on, the targeted sample size was not attained because some targeted individuals were unwilling to participate in the study; therefore we did not meet the intended target. Similarly, the target sample size for animals was not attained. This was because part of the sampling time frame coincided with the rainy season and this rendered the flood plains inaccessible due to flooding. Furthermore, there was not enough time to wait for more persons to be recruited into the study, considering the time frame within which the study had to be accomplished. Because of the reduced sample size,

generalization of these results to other districts is limited. Despite these shortfalls the study generated important information to understand the general epidemiological picture on *Cryptosporidium* infection in resource poor livestock keeping communities.

Hence there is need for further systematic research where a larger sample size would be employed in order to help establish a better understanding of *Cryptosporidium* in the adult HIV infected individuals in Namwala district.

5.4 Recommendations

The following recommendations are based on the above findings:

- Prevalence of *Cryptosporidium* infection in the adult HIV positive population: Based on the findings, there is need for routine screening for *Cryptosporidium* spp. in these patients by the health personnel because most patients were asymptomatic but may act as a source of infection for the population at large.
- Professional risk: Since *Cryptosporidium* spp. infection is prevalent in both humans and animals, medical personnel and care givers attending to the HIV positive population need sensitization. With the prevalence of the infection being high in males than females, there is need to sensitize this risk group (males) in Namwala district because of their frequent contact with livestock. The veterinary officers who attend to livestock will also need sensitization. Sensitization is to be done so as to reduce the spread of infection.
- Marital status: With the high prevalence observed in the divorced and the widowed groups, there is need for a qualitative study to understand the underlying factors that increase the risk of exposure in these categories. Further, future studies should consider equal representation of different groups under marital status. This is because most past studies that looked at marital status showed that being married was a risk factor to *Cryptosporidium* infection without disaggregating the results further into the various marriage categories.

- Prevalence of *Cryptosporidium* infection in cattle: This calls for public awareness programs and also preventive and control measures to be put in place in order to reduce the risk of human exposure as well as animal infections. These public awareness programs can be facilitated by Ministry of Health and Ministry of Fisheries and Livestock.
- Relationship between animal and human infections: Molecular characterization is necessary to identify the *Cryptosporidium* isolates common in both humans and cattle in Namwala district.

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Annexes

Annex I: Information sheet and Informed Consent Form

UNIVERSITY OF ZAMBIA

SCHOOL OF MEDICINE

PUBLIC HEALTH DEPARTMENT

INFORMATION SHEET

Study Title: Prevalence and Factors Associated with Cryptosporidiosis among Adult HIV Positive Population In Contact With Livestock in Namwala District, Zambia – Concurrent Cross Sectional studies.

Principle Investigator:

This Informed Consent Form has two parts:

- Information Sheet (to share information about the research with you)
- Certificate of Consent (for signatures if you agree to take part.

You will be given a copy of the full Informed Consent Form.

Part I: Information Sheet

Introduction

This study is being done by Ntazana Nana Sinyangwe who is currently pursuing her Master's Degree. It will be done as partial fulfilment of the requirements for the degree of Master of Public Health- Environmental Health.

We are doing research on cryptosporidiosis, a diarrheal disease which is common in HIV/AIDS individuals. I am going to give you information and want to invite you to be part of this research. You will be able to read along using the copy that you have been given and are free to ask questions. When all your questions have been answered, you decide whether or not you will participate in the research. You do not have to decide today, and before you make the decision, you can talk to anyone you feel comfortable with about the research.

Purpose of the research

As already mentioned, cryptosporidiosis is a diarrheal disease which is not checked for in routine medical examinations, hence it is usually not listed as a possible cause in diarrheal cases. This disease can be transmitted from animals to human beings and vice versa through contaminated water, food and soil. This disease causes high levels of sickness and death while treatment to reduce this is available. The reason for this study is to find out the burden of this diarrheal disease in the HIV/AIDS population. We also want to know factors that may be associated with its occurrence and also find out the exact species causing this diarrheal disease in Namwala district. Knowledge from this research will help in better management of the disease among the highly affected population in society.

Participant selection

Participants in this study will include all HIV positive individuals aged 18 years and above who are currently receiving ART at Central Namwala District Hospital, Maala and Chitongo Rural Health Centers. The study will not include those who have lived in Namwala for less than 6 months, pregnant women and persons taking drugs which are prescribed for this disease. You have been asked to participate in this study because you fit these descriptions.

Voluntary participation

Your taking part in this research is voluntary and you are free to participate or not. Whether you choose to participate or not, you will still receive all the services offered at this health center. In case you change your mind, you are free to not take part even if you agreed earlier.

Procedures

If you agree to participate, we will ask you to take part in an interview using our close ended questionnaire which has 3 sections on demographic factors, environmental factors and general farm and farm management practices. The last section will only be answered if you have animals in your household. We will also ask you to provide us with a stool specimen in the plastic container provided to check if you have the disease. This will be done after private counseling from the health personnel and agreeing to take part.

Lastly, with your consent we would like to take fecal samples from some of your animals to check the disease status of your animals. Your name will not be included on the questionnaire and sampling containers, instead they will be replaced with numbers.

Risks/ Discomforts

We do not expect any risks, however, there may be some discomfort during disclosure of some of the information asked for during the interview and also during specimen collection. In order to minimize this, the interviewer will work with the counselor to help you understand and go through the study. Gloves, plastic containers and black plastics will be provided to you to collect the samples. We would like to assure you that the information we get from you will not be shared with anyone outside the research team.

Benefits

The only direct benefit is if you test positive for the disease as treatment will be offered which is available at the health centers. Indirect benefits are that the burden of the disease in the district will be known and this information will be passed on to policy makers who will implement the treatment and control strategies.

Payments

There will be no money being given for taking part in this study. However, by participating in the study, you will be treated if found with the disease and also you will contribute to information concerning the disease. This information will help the District Medical Offices to consider this disease as they carry out clinical examinations to reduce morbidity and mortality due to this disease.

Confidentiality

The information that we collect from this research project will be kept confidential. We will protect the information we will get from you by putting it away and no one but the researchers will be able to see it. We will not put names on any information collected from you. Instead, we will use numbers for identification. The number will not be shared with or given to anyone except the medical doctor in- charge in case you are found with the disease under study. As for the stool specimen provided, it will bear the number assigned to you. This specimen will be destroyed as soon as the study is completed.

Sharing the results

The knowledge that we get from doing this research will be shared with you through your counselors. After that, we will publish the results in order that other interested people may learn from our research.

Right to refuse/ withdraw

You are free to decide whether you want to take part in this study or not. You are also free to leave at any point during the interview. You are free not to answer any questions that you are not comfortable with. You are also free not to provide the stool specimen. This will not bring any problem to you as it is your choice and all your rights will be respected.

Who to contact

If you have any questions you may ask them now or during the study. If you wish to ask questions later, you may contact any of the following:

- The principle investigator, Ntazana N. Sinyangwe on +260965931278 or +260977690728 if you have questions or complaints as a result of being in this study.

- ERES CONVERGE IRB office at 33 Joseph Mwilwa road in Rhodes Park, Lusaka if you have questions about your rights as a study participant. You may also contact ERES CONVERGE IRB office if you feel that you have not been treated fairly or if you have other concerns. The ERES CONVERGE IRB office contact numbers are +260955155633/4.

PART II: Certificate of Consent

I have read the information giving or it has been read to me. I have had the chance to ask questions about it which have been answered to my satisfaction. I have voluntarily agreed to take part in this research.

Signature or Participant _____

Date _____

Day/month/year

If illiterate

I have witnessed the accurate reading of the consent form to the potential participant, and the individual has had the chance to ask questions. I confirm that the individual has given consent freely.

Signature of witness _____ AND Thumb print of participant

Date _____

Day/month/year

Statement by the researcher/ person taking consent

I have accurately read out the information sheet to the potential participant, and to the best of my ability made sure that the participant understands that the following will be done:

1. Interview will be conducted using a close ended questionnaire.
2. Stool specimen will be collected from the participant.
3. Stool specimen will be collected from participant's animals.

I confirm that the participant was given the chance to ask questions about the study, and all the questions asked by the participant have been answered correctly and to the best of my ability. I confirm that the individual has not been coerced into giving consent, and the consent has been given freely and voluntarily.

A copy of this Informed Consent Form has been provided to the participant.

Name of Researcher/ person taking the consent _____

Signature of Researcher/ Person taking the consent _____

Date _____

Day/month/year

Annex II: Questionnaire for Human survey

Questionnaire **Serial No** **_____**

A survey to determine the prevalence and factors associated with cryptosporidiosis among the HIV positive population in Namwala District, Zambia.

Please note that all the information will be treated as confidential

Participant's ID: _____ Sex: ___ ____ GPS: _____ Health Center ID: _____

Name of interviewer: _____

Section A: Demographic factors

(Put X where applicable)

1. How old are you?

2. What is the highest level of education attained? (Specify the grade)
    a) None _____
    b) Primary _____
    c) Secondary _____
    d) Tertiary _____

3. Are you in gainful employment? (If yes please specify)
    a) Yes ____ Specify _____
    b) No ____

4. What is your monthly income?
    a. Less than 500 ____

b. Between 500-1000 ____
c. Between 1000-5000 ____
d. Above 5000 ____

Section B: Environmental factors

5. What is your household water source?
    a) Borehole ____
    b) Shallow well ____
    c) Stream/River ____
    d) Municipal supply ____
    e) Other (specify) ____
6. Is the water source,(indicate)
    a) Private ____
    b) Communal ____
7. Do your neighbors get water from your source?
    a) Yes ____
    b) No ____
8. Is the household water treated?
    a) Yes ____
    b) No ____
9. If yes, which method is used?
    a) Chlorination ____
    b) Boiling ____
    c) Other (specify) ____
10. What is your main occupation?
    a) Livestock rearing ____
    b) Farming ____
    c) Trading ____
    d) Other (specify) ____

11. Do you keep any animals?
    a) Yes ____
    b) No ____
12. What type of animals do you keep?
    Specify _____
13. Do any of your neighbors keep animals?
    a) Yes ____
    b) No ____
14. Do you share water source with the animals?
    a) Yes ____
    b) No ____
15. Do you have contact with calves, lambs, kids or piglets?
    a) Yes ____
    b) No ____
16. If yes, what type of contact is it?
    a) Feeding the calves, lambs, kids or piglets ____
    b) Cleaning calf, lamb kid or piglet pens ____
    c) Others (specify) ____
17. Does any of your family members have contact with animals?
    a) Yes ____
    b) No ____
18. If yes, what type of contact is it?
    Specify _____
19. Have you had any diarrheal cases in the house in the last 3 weeks?
    a) Yes ____
    b) No ____
20. If yes, how many family members were affected and what was the duration?
    Specify _____
21. Did the affected person have any prior contact with an affected patient or animal?
    a) Yes ____
    b) No ____

22. Were there any diarrheal cases in the animals in the last 1 month?
    a) Yes ____
    b) No ____
23. If yes, how many were affected and what was the duration?
    Specify _____

Section C: General farm and farm management information

(To be answered by those keeping animals or are farm workers)

24. What type of animals are kept on the farm? (put X)
    a) Cattle ____
    b) Sheep ____
    c) Goats ____
    d) Pigs ____
25. What is the total number of animals?
    a) Cattle ____
    b) Sheep ____
    c) Goats ____
    d) Pigs ____
26. What is the total number of
    a) Calves ____
    b) Lambs ____
    c) Kids ____
    d) Piglets ____
27. Type of husbandry used?
    a) Intensive
    b) Semi- intensive
    c) Extensive
    d) Free range
28. Type of housing Calves Lambs Kids
    Piglets

a) Individual housing ____ ____ ____ ____

b) Group housing ____ ____ ____ ____

29. Type of flooring Calves Lambs Kids Piglets

a) Concrete ____ ____ ____ ____

b) Soil ____ ____ ____ ____

c) Other (specify) ____ ____ ____ ____

30. Type of bedding Calves Lambs Kids Piglets

a) None ____ ____ ____ ____

b) Straw/Hay ____ ____ ____ ____

c) Sand ____ ____ ____ ____

d) Sawdust ____ ____ ____ ____

e) Other (specify) ____ ____ ____ ____

31. How often is the bedding removed or cleaned?

Calves Lambs Kids Piglets

a) Daily ____ ____ ____ ____

b) Several times/week ____ ____ ____ ____

c) Weekly ____ ____ ____ ____

d) Less than monthly ____ ____ ____ ____

e) Monthly ____ ____ ____ ____

32. How are the young animals fed? Calves Lambs Kids Piglets

a) Suckle ____ ____ ____ ____

b) Bottle fed ____ ____ ____ ____

c) Other (specify) ____ ____ ____ ____

33. What is the drinking water source?

a) Shallow well ____

b) Borehole ____

c) Stream/river ____

d) Pool/dam ____

e) Other (specify) _____

34. Is there a diarrheal problem in the (indicate yes or no)

a) Calves ____

b) Lambs ____

c) Kids ____

d) Piglets ____

35. Is there mortality due to diarrhea? (indicate yes or no)

a) Calves ____

b) Lambs ____

c) Kids ____

d) Piglets ____

Thank you for your co-operation

Annex III: Bio-Data sheet for animal survey

Bio-Data Sheet

Entry No	Household ID No	Animal ID No	Age	Sex	Breed

Annex IV: Ethical clearance

33 Joseph Mwilwa Road
Rhodes Park, Lusaka
Tel: +260 955 155 633
+260 955 155 634
Cell: +260 966 765 503
Email: eresconverge@yahoo.co.uk

I.R.B. No. 00005948
E.W.A. No. 00011697

23rd September, 2015

Ref. No. 2015-June-012

The Principal Investigator
Dr. Ntazana Nana Sinyangwe
University of Zambia
Dept. of Public Health – Environmental Health Unit
P.O. Box 50110,
LUSAKA.

Dear Dr. Sinyangwe,

RE: PREVALENCE AND FACTORS ASSOCIATED WITH CRYPTOSPORIDIOSIS AMONG HIV POSITIVE POPULATION IN CONTACT WITH LIVESTOCK IN NAMWALA DISTRICT

Reference is made to your corrections dated 7th September, 2015. The IRB resolved to approve this study and your participation as principal investigator for a period of one year.

Review Type	**Ordinary**	Approval No. **2015-June-012**
Approval and Expiry Date	Approval Date: 23rd September, 2015	Expiry Date: 22nd September, 2016
Protocol Version and Date	Version-Nil	22nd September, 2016
Information Sheet, Consent Forms and Dates	• English.	22nd September, 2016
Consent form ID and Date	Version-Nil	22nd September, 2016
Recruitment Materials	Nil	22nd September, 2016
Other Study Documents	Questionnaire, Bio-data Sheet.	22nd September, 2016
Number of participants approved for study	388	22nd September, 2016

Specific conditions will apply to this approval. As Principal Investigator it is your responsibility to ensure that the contents of this letter are adhered to. If these are not adhered to, the approval may be suspended. Should the study be suspended, study sponsors and other regulatory authorities will be informed.

Conditions of Approval

- No participant may be involved in any study procedure prior to the study approval or after the expiration date.
- All unanticipated or Serious Adverse Events (SAEs) must be reported to the IRB within 5 days.
- All protocol modifications must be IRB approved prior to implementation unless they are intended to reduce risk (but must still be reported for approval). Modifications will include any change of investigator/s or site address.
- All protocol deviations must be reported to the IRB within 5 working days.
- All recruitment materials must be approved by the IRB prior to being used.
- Principal investigators are responsible for initiating Continuing Review proceedings. Documents must be received by the IRB at least 30 days before the expiry date. This is for the purpose of facilitating the review process. Any documents received less than 30 days before expiry will be labelled "late submissions" and will incur a penalty.
- Every 6 (six) months a progress report form supplied by ERES IRB must be filled in and submitted to us.
- ERES Converge IRB does not "stamp" approval letters, consent forms or study documents unless requested for in writing. This is because the approval letter clearly indicates the documents approved by the IRB as well as other elements and conditions of approval.

Should you have any questions regarding anything indicated in this letter, please do not hesitate to get in touch with us at the above indicated address.

On behalf of ERES Converge IRB, we would like to wish you all the success as you carry out your study.

Yours faithfully,
ERES CONVERGE IRB

Mrs. M. Mayamiko-Mbewe
RNM, DNE, BSc., M.Ed.
ACTING CHAIRPERSON

Annex V: Approval letter for conducting the study

THE NATIONAL HEALTH RESEARCH AUTHORITY
C/O Ministry of Health
Ndeke House
P.O. Box 30205
LUSAKA

In reply please quot

No.....

MH/101/23/10/1

29th October, 2015

Dr N Sinyangwe
University of Zambia
P.O Box 50110
LUSAKA

Re: Request for Authority to Conduct Research

The National Health Research Authority is in receipt of your request for authority to conduct research titled **"Prevalence and Factors Associated with Cryptosporidiosis among HIV Positive Population in Contact Livestock in Namwala District"**.

I wish to inform you that following submission of your request to the Authority, our review of the same and in view of the ethical clearance, this study has been approved to carry out the above mentioned exercise on condition that:

1. The relevant Provincial and District Medical Officers where the study is being conducted are fully appraised;
2. Progress updates are provided to NHRA quarterly from the date of commencement of the study;
3. The final study report is cleared by the NHRA before any publication or dissemination within or outside the country;
4. After clearance for publication or dissemination by the NHRA, the final study report is shared with all relevant Provincial and District Directors of Health where the study was being conducted, and all key respondents.

Yours sincerely,

Dr. P. Chanda-Kapata
For/Director
National Health Research Authority