PREVALENCE AND ASSOCIATED FACTORS FOR *CRYPTOSPORIDIUM* INFECTION IN CALVES AND ENVIRONMENT IN ASEMBO RARIEDA, SIAYA COUNTY-KENYA

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Prevalence and Associated Factors for *Cryptosporidium* Infection in Calves and Environment in Asembo Rarieda, Siaya County-Kenya

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other university

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DEDICATION

This thesis is dedicated to the almighty God for having granted me life, good health, and strength, my late father and mother for having left fingerprints of grace in our lives, my step mother - Nyasembo, my five brothers and twelve sisters and most importantly my family-my wife Judith and our children Nadia, Teannah, Johnson and Javan, for giving me easy moments and encouraging me during my studies and for their kindness and devotion.

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TABLE OF CONTENTS

LIST OF TABLES

TABLE OF FIGURES

LIST OF APPENDICES

ACRONYMS AND ABBREVIATIONS

AIDS	Acquired Immunodeficiency Syndrome
ARV	Anti-Retroviral Therapy
CDC	Canters for Disease Control and Prevention
DNA	Deoxyribonucleic Acid
ERC	Ethics Review Committee
HAART	Highly Active Antiretroviral Therapy
HIV	Human Immunodeficiency Virus
KEMRI	Kenya Medical Research Institute
MOH	Medical Officer of Health
mZN	Modified Ziehl-Neelsen
PBIDS	Population Based Infectious Disease Surveillance
SCVO	Sub County Veterinary officer
SRC	Scientific Review Committee

OPERATIONAL DEFINITION OF TERMS

Diarrhea	Loose and watery feces
Heaped Manure	Cattle feces collected and piled at one point for other uses such as fertilizer or smearing mud houses in rural set ups
Immunocompromised	Weakened immunity
Surface run-off	Flow of water occurring on the ground surface when excess rainwater or other sources can no longer sufficiently rapidly infiltrate in the soil

ABSTRACT

Cryptosporidium species can infect a wide range of animals including birds, reptiles, fish, cattle and man. These zoonotic enteric coccidian parasites are among the leading causes of diarrhea in calves (less than six months old) and children (less than five years old). These parasites contribute substantially to morbidity and mortality in both cattle and young children in resource-poor countries. About 20% of diarrhea in under-fives worldwide can be attributed to cryptosporidiosis. The disease is routinely diagnosed using microscopy by recognition of acid-fast oocysts in stool. Infective oocysts can survive for long periods in the environment because they are resistant to common household disinfectants and there is no specific treatment against cryptosporidiosis in man and animals. In Kenya, there is very little information on cryptosporidiosis since few studies have been conducted on this organism. In Rarieda Sub County, the prevalence of *Cryptosporidium* infection both in the environmental slurry and calves is not known yet there is a high prevalence of HIV and Cryptosporidiosis is one of the main opportunistic infections. A study conducted among children in the same area reported high prevalence of *Cryptosporidium* hence the need to find out the level of prevalence in animals and environment. The aim of this investigation was to determine the level of oocyst contamination in the environment, the prevalence in calves aged six months and below and also to find out the factors associated with infection in calves. This was a cross-sectional study wherein 350 calves aged six months and below were randomly selected using simple random sampling technique and 187 samples were collected randomly from heaped manure in these households. Diagnosis was done using the modified Ziehl Nelseen staining technique. Data on potential associated factors was collected by use of a pre-tested structured questionnaire. The study detected a prevalence of 8.3% (95% CI: 5.7-11.8) in the calves and 7.5% (95% CI: 4.2-12.2) in the environmental samples collected. Among the calves, a higher prevalence rate was reported during the rainy season than the dry season at 12.5% and 6.09% respectively (OR=2.198, 95% C.I 1.011- 4.801, p= 0.043). Odds of infection were also higher in diarrheic calves compared to those with normal stool (AOR=6.1, 95% C.I 2.2-16.9, $p<0.05$), calves aged ≤2 months old compared to older calves (AOR=12.7, 95% C.I: 4.5-35.8) and calves raised in poor sanitation compared to calves in good hygienic conditions (AOR=9.9, 95% C.I: 3.1-30.7) . Of all the people interviewed, 70% were not aware of any zoonotic disease transmissible to them through contact with animal feces/manure. Of the 350 households, 83.71% have their water sources not restricted from animal access and 68.00% have children less than five years of age. In terms of frequency of contact with animals and children, 50.21% of the children had frequent close contact with the cattle. For housing of calves, 32% are housed in the kitchen at night, 29.43% have calf pens, and 23.14% sleep in cow sheds while 15.43% sleep in the open. The overall prevalence and distribution of *Cryptosporidium* spp. infection was associated with presence of diarrhea in calves. Calves sleeping in the kitchens, accessing water used by humans and improper handling of manure pose higher risk for human

infection. In resource-poor settings, environmental sampling can be used as a proxy indicator to assess prevalence of *Cryptosporidium* Spp. in calves and prevention interventions should be targeted at younger calves since they are at higher risk of infection. Public education on importance of maintaining high hygiene standards when handling manure remain the most effective preventive measure.

CHAPTER ONE

INTRODUCTION

1.1 Background

Cryptosporidium species are coccidian parasites of the phylum Apicomplexa known to cause diarrhea in humans and animals globally. These coccidian parasites of the genus *Cryptosporidium* can infect a wide range of animals, including, birds, cattle, reptiles and even fish (Xiao, Fayer, Ryan, & Upton, 2004). Over the last 30 years, *Cryptosporidium* species infection has continued to gain public health importance as a major cause of diarrhea in humans, especially children below five years and calves under six months and has been classified as an emerging pathogen by the Centre for Disease Control (CDC) (Ronald Fayer, Morgan, & Upton, 2000). Currently, there are 22 valid species of *Cryptosporidium* that are known to be infective to vertebrate hosts but out of these, only 13 species are considered valid by most investigators (Ramirez, Ward, & Sreevatsan, 2004). Out of these 13 species, *C. parvum* and *C. hominis* are the most important species due to their widespread distribution (Fayer R, 2010). *Cryptosporidium parvum* has been reported as the main zoonotic species that affects cattle and as the major cause of diarrhea in calves and children worldwide (Plutzer & Karanis, 2009b). *Cryptosporidium hominis* is maintained in human-to-human cycles (Swai & Schoonman, 2010), but has been detected in calves in Nairobi, Kenya hence considered anthroponotic (Kang'ethe *et al.*, 2012). Various other Cryptosporidium species which have been detected by other researchers in human feaces include; *C. meleagridis* (Akiyoshi *et al*., 2003), *C. felis* (Cacciò, De Giacomo, & Pozio, 2002; Coupe, Sarfati, Hamane, & Derouin, 2005), *C. canis* (Coupe *et al.*, 2005), *C. muris* (Morgan *et al.*, 2000) and *C. baileyi* (Ditrich *et al.*, 1991) indicating possible infectivity to humans, mainly in immunocompromised individuals. Humans are thought to acquire the parasite by ingestion of oocysts, which are shed in the stool of infected animals or other humans. Cryptosporidium oocysts have been found to contaminate different water sources including swimming pools (Leav, BA, M, & HD., 2003).

Contaminated manures from cattle can be major sources of *Cryptosporidium* oocysts unless proper manure management or treatment strategies are used to minimize oocyst viability or transport to water sources (Kuczynska & Shelton, 1999). In addition to direct fecal deposition, possible modes of transport to potable or recreational water include surface transport from land-applied manures or leaching through the soil to groundwater (Kuczynska & Shelton, 1999). Runoff from the land contaminated by these sources might serve as a vehicle through which the *Cryptosporidium* oocysts can travel into water sources. Thus farms that keep cattle may serve as a potential source of *Cryptosporidium* exposure to human populations.

The commonly used method of detection of *Cryptosporidium* oocysts is through examination of acid-fast stained stool for presence of oocysts which measure 4-6 µm with spherical appearance.

In Kenya few studies have been carried out to determine the prevalence and possible zoonotic potential of *Cryptosporidium* Spp. both in cattle and children. A study conducted in children in Kenya detected a 4% prevalence of *Cryptosporidium* infection and reported *Cryptosporidiosis* as main cause of diarrhea. The species isolated in that study included; *C.hominis, C.parvum, C.canis, C.felis, C.meleagridis,* and *C.muris*. This clearly shows that the sources of these infections were animals since most of these species are known to be specific to animals (Gatei *et al.*, 2006). Another study in children and animals in Ethiopia reported a nearly equal prevalence of *Cryptosporidium* Spp. infection in animals and children (Wegayehu, Adamu, & Petros, 2013), showing that in resource-poor settings, the detected prevalence of *Cryptosporidium* reported in the animals can be used as a proxy indicator of the level of infection in humans. Contaminated water sources and infected animals have been known to be a major source of human infections. Once humans are infected, sequential infections can be maintained through person to person transmission (Chako, Tyler, Schultz, Chiguma & Beerntsen, 2010). Since there is no known treatment for Cryptosporidiosis yet it has been proved to be zoonotic and life-threatening in immunocompromised individuals, its control and prevention requires a multi-disciplinary approach with close collaboration between animal health and

human health sectors, making it a disease of immense public health importance. In Kenya, Cryptosporidiosis was ranked among the top twenty priority zoonotic diseases based on its severity of illness, epidemic potential, socio-economic impact and unavailability of known medical intervention thus increasing the need to further investigate its prevalence in potential reservoirs for human infection (Munyua *et al*., 2016).

This study aimed to determine the prevalence of *Cryptosporidium* Spp. in calves aged below six months and the level of environmental contamination in these farms and to establish the factors associated with *Cryptosporidium* Spp. positivity in calves. Since humans always have close contact with cattle and cattle manure, this might indicate the potential risk posed to the humans by the cattle.

1.2 Statement of the Problem

Over the last 30 years *Cryptosporidium* infection has continued to gain public health importance as an emerging zoonotic infection. This is because of the increasing population of the immunocompromised persons. The major conditions leading to immunosuppression include HIV/AIDS and malnutrition (Cabada & White, 2010). Around 1.3 million people die of the consequences of diarrhea globally every year (Naghavi *et al*., 2015). *Cryptosporidium* Spp. has been identified as one of the six major pathogens responsible for diarrhea in children younger than 5 years in Africa and Asia (Kotloff *et al*., 2013). In the Global Burden of Disease 2016, *Cryptosporidium* infection was the fifth leading diarrhoeal aetiology in children younger than 5 years, and acute infection caused more than 48 000 deaths globally and more than 4·2 million disability-adjusted life-years lost (Global Burden of Disease Collaborative Network., 2017).

Various studies on HIV patients have reported an alarming high prevalence of *Cryptosporidium* Spp., showing that Cryptosporidiosis is a major cause of diarrhea in immunocompromised individuals (Girma, Teshome, Petros, & Endeshaw, 2014). It is worth noting that a single oocyst is sufficient to cause infection and disease in

susceptible hosts (Pereira, Ramirez, Xiao, & Ward, 2002) hence the importance of determining the prevalence in calves and manure, which act as reservoirs.

In humans, it accounts for up to 20% of all cases of childhood diarrhea in the developing countries whereas its true burden in animals is not known (Ronald Fayer *et al.*, 2000). Various studies in Eastern Africa region have reported prevalence of *Cryptosporidium* Spp. in animals ranging from 2.2% to 35% in cattle (Salyer, Gillespie, Rwego, Chapman, & Goldberg, 2012; Swai & Schoonman, 2010).

A recent multi-centre study determining the burden and aetiology of diarrheal disease in infants and young children in Sub-Saharan Africa and Asia identified *Cryptosporidium* to be the second major cause of diarrhea in children aged 0-11 months after rotavirus (Kotloff *et al*., 2013). This study was conducted among children aged below five years. Among the various study sites, we had Asembo in Rarieda Sub County. Since this study established prevalence of *Cryptosporidium* infection in children, this study sought to determine the prevalence of the same in calves and in manure.

Studies on Cryptosporidiosis conducted in Kenya and other parts of the world have mainly been carried out on humans. In humans, the most prevalent *Cryptosporidium* species has been reported to be *C. hominis* followed by *C. parvum*. The latter is the most prevalent in cattle and hence the main zoonotic species of *Cryptosporidium*. There are very few studies on calves and other livestock. *Cryptosporidium* Spp. is transmitted primarily by direct contact with feces of humans or animals (Zambrano, Levy, Menezes, & Freeman, 2014). Other studies have shown transmission to occur through other routes, such as drinking of contaminated water and eating of fresh produce that has been fertilized with manure or irrigated with contaminated water. Contamination of crops, other agricultural products, and surface water with feces from cattle and other livestock, used as organic manure, therefore serves as an important mechanism of zoonotic transmission to humans (Shirley, Moonah, & Kotloff, 2012). This creates a need to determine the level of manure contamination with Cryptosporidium oocysts in order to quantify the risk posed to humans and livestock.

A better understanding of the prevalence of *Cryptosporidiosis* in animals, environment and its associated risk factors may help in tailoring specific control programs that will suit these particular situations and these, if implemented may reduce the impact and incidences of the infection in both livestock and humans. This study aimed to determine the prevalence of *Cryptosporidium* in Calves, in manure and assess the factors associated with infection in claves since infected calves are considered to be an important reservoir of zoonotic *Cryptosporidium* and the close contact between humans and animals increases this risk. Apart from the zoonotic risk, the infection of livestock with *Cryptosporidium* often results in decreased production and loss of income for the livestock sector (Sweeny, Ryan, Robertson, & Jacobson, 2011)

1.3 Significance of the Study

Cryptosporidium spp. have a low infective threshold, are resistant to common household disinfectants such as chlorine and thus have the potential of surviving and persisting in the environment for a long period of time. Therefore, an understanding of the prevalence of the parasite in calves and in the environment could estimate the threat for zoonotic infection to humans due to the close contact and human practices that predispose them to infection. Also, calves living in close proximity to rivers have a potential to contaminate the waters through surface run-offs hence a potential cause of water-borne infections to humans.

1.4 Study Justification

There is very little information on the prevalence of *Cryptosporidium* Spp. infection in cattle in Kenya and more so, the zoonotic potential and epidemiologic relationship between the species in human and animals. Veterinary reports show diarrhea as a major cause of morbidity and mortality in calves yet the actual cause of the diarrhea is usually diagnosed clinically and not laboratory confirmed. *Cryptosporidium parvum,* the main zoonotic species, is a common cause of calf diarrhea.

Cryptosporidium Spp. are perfectly adapted to infect human beings through various routes of transmission such as; zoonotic, foodborne, water-borne and human-to-human.

This study therefore aimed to investigate the prevalence of *Cryptosporidium* oocysts among calves and the level of environmental contamination by *Cryptosporidium* oocysts in Asembo, Rarieda Sub County- Siaya County since the infected calves and contaminated manure, coupled with the close contact between humans and livestock, may pose a high risk of zoonotic infection.

It is important to determine the prevalence of *Cryptosporidium* in the environment and in calves since the infected calves and contaminated manure remain potential reservoirs for zoonotic *Cryptosporidium* that can be easily transmitted to household members and farm workers, in cattle keeping societies, if proper hygiene and calf management practices are not followed. The oocysts can also contaminate milk and drinking water (Muchiri *et al*., 2009). Various studies on HIV patients have reported an alarming high prevalence of *Cryptosporidium* Spp., showing that Cryptosporidiosis is a major cause of diarrhea in immunocompromised individuals (Girma *et al*., 2014).

Asembo has a high prevalence of HIV and therefore providing more justification to conduct the study there since *Cryptosporidium* Spp. is among the leading causes of opportunistic infections in HIV patients. It is worth noting that a single oocyst is sufficient to cause infection and disease in susceptible hosts hence the importance of determining the prevalence in calves and manure, which act as reservoirs (Pereira *et al*., 2002). By determining the prevalence in calves and in heaped manure, public health information on control strategies can then be targeted towards the population at risk of zoonotic infection thus curtailing the spread to humans since there is no effective medication against Cryptosporidiosis.

Various outbreaks of *Cryprosporidium* Spp. infection have been reported in many parts of the world and these have been attributed to drinking contaminated water. Most importantly is the massive outbreak in Milwaukee which occurred through contaminated water supply (Mac Kenzie *et al*., 1994).

Other studies have demonstrated the danger of transmission of *Cryptosporidium* oocysts to humans through contaminated animal manure and therefore, understanding the environmental pathways of *Cryptosporidium* through contaminated manure is essential for effective management and control of human and animal *Cryptosporidium* infection (Vermeulen, Benders, Medema, & Hofstra, 2017).

The findings of this study will provide information on prevalence and risk factors of *Cryptosporidium* spp. in calves and heaped manure thus provide appropriate recommendations which will be used to guide evidence-based interventions geared towards prevention and control of *Cryptosporidiosis* in both animals and humans.

1.5 Research questions

1. What is the prevalence of *Cryptosporidium* infection in calves below 6 months of age in Asembo Rarieda, Siaya County?
2. What is the prevalence of *Cryptosporidium* oocysts in heaped manures in Asembo Rarieda, Siaya County?
3. What are the factors associated with *Cryptosporidium* infection in calves aged below 6 months?

1.6 Objectives

1.6.1 General objective

To determine the prevalence and associated factors of *Cryptosporidium* infection in calves aged 6 months and environment in Asembo Rarieda Sub County-Siaya County

1.6.2 Specific objectives

- To determine the prevalence of *Cryptosporidium* infection in calves aged below 6 months in Asembo Rarieda, Siaya County
- To establish the prevalence of *Cryptosporidium* oocysts in heaped manure in Asembo Rarieda, Siaya County
- To determine the factors associated with *Cryptosporidium* infection in calves below 6 months of age

1.7 Conceptual Framework

The conceptual framework for this study is as below:

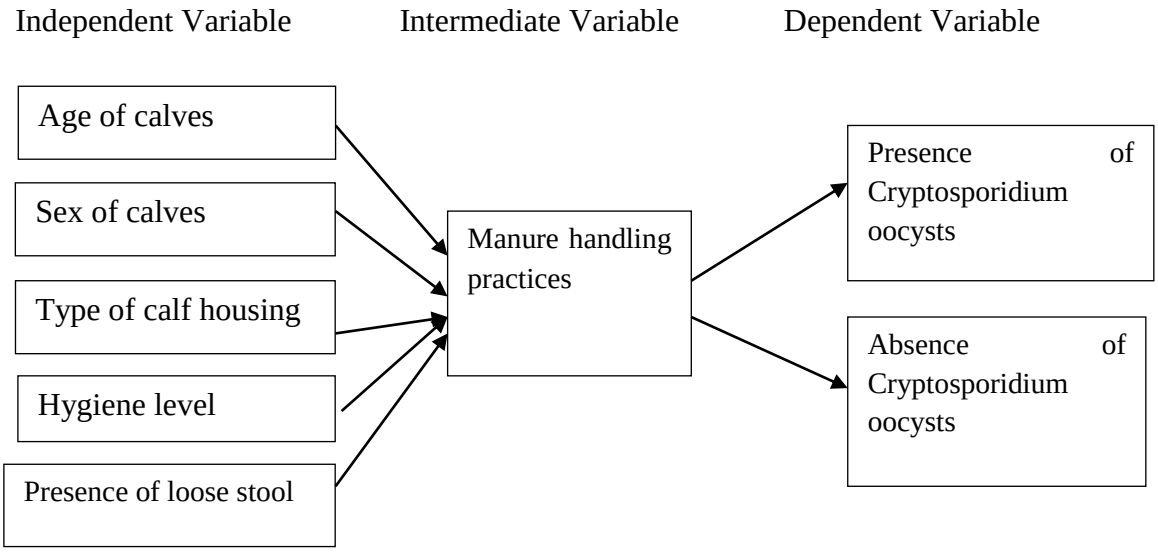

Figure 1.1: Conceptual Framework

CHAPTER TWO

LITERATURE REVIEW

2.1 Historical Background

Cryptosporidium genus was first discovered in 1907 by a scientist known as Ernest Edward Tyzzer (Tzipori, & Ward, 2002). He isolated *Cryptosporidium muris* from the gastric glands of laboratory mice. He later published a complete description of the life cycle and subsequently described a second species known as *C. parvum*, also from laboratory mice in 1912. Tyzzer distinguished this second species from *C. muris* by experimentally infecting mice and showing that *C. parvum* was smaller and developed only in the small intestine rather than the stomach. Tyzzer was first to report on avian Cryptosporidiosis in 1929 (Tzipori, & Ward, 2002). Interest in *Cryptosporidium* (C. parvum) by the veterinary medical profession was stimulated in 1971 when this protozoan was first reported to be associated with diarrhea in cattle (Ramirez *et al.*, 2004). Thereafter, numerous case reports from many different animals are now present in the literature and one species, *C. parvum*, is recognized as an important cause of neonatal diarrhea in calves and lambs (Trotz-Williams *et al.*, 2007) . Another species, *C. baileyi*, is now recognized as an important cause of respiratory disease in poultry (da Cunha, Cury, & Santín, 2018). Cryptosporidiosis in calves was recognized in the 1970s (Anderson, 1998).

The first case of Cryptosporidiosis in humans was described in 1976 in a three-and-a-half-year-old girl who developed a self-limiting enterocolitis. But it was not until *Cryptosporidium* infections were reported as a cause of death in HIV/AIDS patients in the 1980s that the protozoan parasite was accepted as a significant zoonotic pathogen warranting scientific research in humans (Wang *et al*., 2018).

Following the initial discovery of *Cryptosporidium*, over 50 years elapsed during which the parasite was commonly confused with other apicomplexan genera, especially members of the coccidian genus *Sarcocystis*. This was because many *Sarcocystis* spp. have oocysts with thin walls that often rupture, releasing free

sporocysts, and each sporocyst contains four sporozoites like *Cryptosporidium* oocysts. Subsequent ultra-structural studies confirmed the endogenous stages of *Cryptosporidium* spp. to possess a unique attachment organelle - which is the key feature that currently defines the genus and family (Xiao *et al.*, 2004).

Cryptosporidium sparked great public health interest after the large human waterborne outbreak in Milwaukee in 1993, and rapidly became recognized as one of the most serious and difficult waterborne pathogens to control. Subsequent reports have demonstrated its worldwide distribution and zoonotic potential (Gupta & Haas, 2004).

Over time, the perception of *Cryptosporidium* spp. has changed from that of a rare opportunistic pathogen to that of an important worldwide cause of diarrheal illness in humans and domesticated animals (Anderson, 1998).

2.2 Taxonomy of Cryptosporidium species

Cryptosporidium is one of the several protozoan genera in the phylum Apicomplexa (Xiao *et al*., 2004). They are all referred to as coccidian. *Cryptosporidium* is oval-shaped and can be found in a wide range of hosts including; man, mammals, birds, fish and reptiles. The parasite replicates intracellularly in the brush border of the small intestines. Infective oocysts are shed into the lumen and passed in the feces (Tzipori, & Ward, 2002).

At least 22 species of cryptosporidium have been named based on host occurrence, parasite morphology, host predilection and site of infection. However, only 13 species are considered valid by most investigators (Ramirez *et al*., 2004). These include *C. andersoni* (cattle), *C. baileyi* (chicken and some other birds), *C. canis* (dogs), *C. felis* (cats), *C. galli* (birds), *C. hominis* (humans), *C. meleagridis* (birds and humans), *C. molnari* (fish), *C.muris* (rodents and some other mammals), *C. parvum* (ruminants and humans), *C. wrairi* (guinea pigs), *C. saurophilum* (lizards and snakes), and *C. serpentis* (snakes and lizards).

Table 2.1: Taxonomic classification of Cryptosporidium (Ghazy, Abdel-Shafy, & Shaapan, 2015)

Category	Name
Phylum:	Apicomplexa
Class:	Sporozoasida
Subclass:	Coccidiasina
Order:	Eucoccidiorida
Suborder:	Eimeriorina
Family:	Cryptosporidiidae
Genus:	*Cryptosporidium*

2.3 Life Cycle

The life cycle of Cryptosporidium begins as sporulated oocysts which are released to the environment through feces of infected hosts. The oocysts can survive for long periods in the environment since it is resistant to many common household disinfectants, especially chlorine-based (Bogan, 2018).

The life cycle of *Cryptosporidium* is illustrated in Figure 2.1

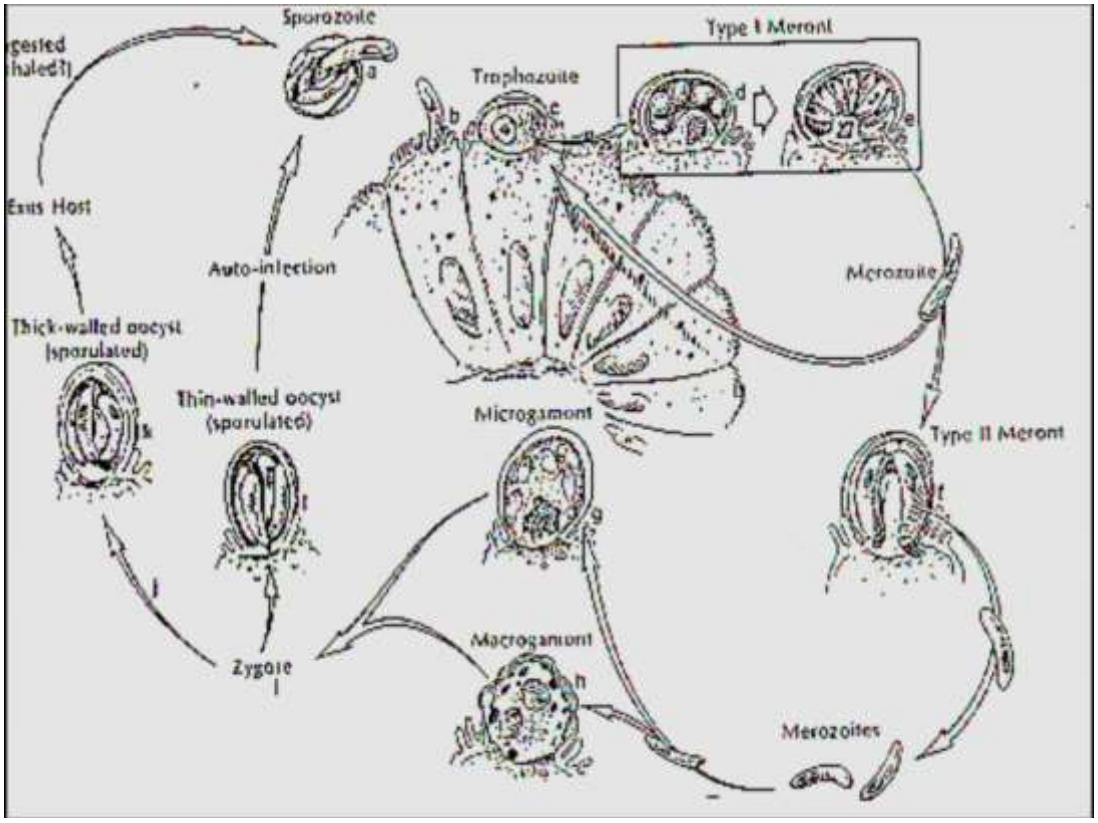

Figure 2.1: Life cycle of Cryptosporidium (Current & Blagburn, 1990)

The life cycle consists of an asexual stage and a sexual stage. The sporulated oocyst is the only exogenous stage and is excreted through feces of an infected host. The endogenous phase begins after a suitable host ingests the oocyst. When the oocysts are ingested by the host animal, they excyst to release sporozoites which attach to the microvilli of the epithelial cells of the small intestines and respiratory tracts. Each oocyst contains four sporozoites and these are the infective stages. Excystation of sporozoites require exposure to pancreatic enzymes and/or bile salts. However, these exposures only enhances excystation but sporozoites can also excyst in warm aqueous solutions alone and this explains the ability of Cryptosporidium to infect extra-intestinal sites such as respiratory tract, conjunctiva of the eye, gall bladder, lymph nodes, testicles, uterus and vagina.

The sporulated oocyst is the only exogenous stage and is excreted through feces of an infected host. The endogenous phase begins after a suitable host ingests the oocyst.

Sporozoites and subsequent developmental stages are all found at the luminal surface of the epithelium. The microvilli surround the sporozoite thus making it intracellular but extra-cytoplasmic. At the interface of the parasite and the host cell, each stage has a "feeder" or" attachment" organelle which is unique to the genus *Cryptosporidium.* The function of the organelle has not yet been determined but appears to increase the surface area between the parasite and host cell hence facilitating exchange of material.

Each sporozoite differentiates into spherical trophozoites. Asexual multiplication known as schizogony or merogony, results when the trophozoite nucleus divides. *C. baileyi* has 3 types of schizonts or meronts and *C. parvum* has 2 types. For *C. parvum* Type 1 schizonts develop six or eight nuclei and each is incorporated into a merozoite a stage structurally similar to the sporozoite (Tandel *et al.*, 2019).

Each mature merozoite leaves schizonts to infect another host cell and develop into another Type 1 or Type 2 schizonts that produce 4 merozoites. It is thought that only merozoites from type 2 schizonts initiate sexual multiplication (gametogony) upon infecting new host cells by differentiating into either microgamont (male) or macrogamont (female) stage. Each microgamont becomes multinucleate and each nucleus is incorporated into microgamete, a sperm cell equivalent. Macrogamont remains uninucleate, an equivalent of an ovum. It is assumed that only fertilized macrogamonts develop into oocyst that sporulate in situ and contain 4 sporozoites. Oocysts in the intestinal tract are excreted with feces whereas those in the respiratory tract exit the body with the respiratory or nasal secretions (Tandel *et al*., 2019). Some literature suggest that oocysts with thin walls release sporozoites that autoinfect the host whereas those with thicker walls leave the body to infect other hosts (Heo *et al.*, 2018) . The prepatent period is the shortest time after ingestion of infective oocyst for the parasite to complete the endogenous life cycle and excrete newly developed oocyst. This time varies with the host and species of *Cryptosporidium.*

Experimentally determined prepatent periods for *C. parvum* ranges from two to seven days for calves, two to fourteen days for dogs, three to six days for pigs, two to five days for lambs and four to twenty two days for humans The patent period is the duration of oocyst excretion experimentally determined for *C. parvum* range from 1 to 12 days for calves, 3 to 33 days for dogs, 5 to 14 days for pigs and 1 to 20 days for human (Gerace, Presti, & Biondo, 2019).

2.4 Occurrence and Distribution

Cryptosporidium is a public health- important and widely distributed enteric pathogen of young livestock and humans and it's common in other hosts but often asymptomatic. It causes acute self-limiting gastroenteritis in immunocompetent individuals and persistent and potentially fatal infection in the immunocompromised worldwide (Plutzer & Karanis, 2009a). It has a wide host range and isolates from mammals have been successfully transmitted to both homologous and heterologous host species. Because the parasite is not host-specific, infections in other agricultural animals, rodents, wildlife species and companion animals are regarded as potential reservoirs of infection both for livestock and humans.

Most data on the incidence of *C. parvum* infection in domestic animals which have been recorded worldwide have been reported in cattle (Anderson, 1998). Cryptosporidium have been reported to cause several water-borne and food-borne outbreaks worldwide (Ronald Fayer *et al.,* 2000). Cryptosporidiosis has been recognized as a common gastrointestinal problem in calves in the United Kingdom (Brook, Hart, French, & Christley, 2008). The U.K. Veterinary Investigation Service diagnosed 2,177 episodes of infection in calves in 1994 versus 216 in 1984. While this is not the total number of animals affected it reflects samples from sites where one or more calves were infected at a specific time.

There is little data on prevalence for infection in domestic dogs. Individual cases in young dogs have been reported, often with concurrent infection such as canine distemper virus (Rosanowski, Banica, Ellis, Farrow, Harwood. Jordan, & Blake, 2018). In Kenya, reports on cryptosporidiosis, both in man and animals, remain

scanty and few studies have been done to assess the prevalence of the disease. A study conducted in Kenya among children residing in informal settlements reported a prevalence of 36.7%.(Mbae *et al*., 2013). Another study reported a prevalence of 4% in children under five years (Gatei *et al.*, 2006). In cattle, a study conducted in Kenya reported an overall prevalence of 7% (Kang'ethe *et al*., 2012)

In a survey carried out in Scottland to identify the species and genotypes of Cryptosporidium in drinking and raw water, three species namely; *C. andersoni*, *C. parvum* and *C. ubiquitum* were detected (Nichols, Connelly, Sullivan, & Smith, 2010). Another study in Kenya reported C. parvum and *C. andersoni* in watersheds that are shared by wildlife, domestic animals and humans (Muchiri *et al*., 2009).

2.5 Diagnosis of Cryptosporidium

Testing for *Cryptosporidium* is not always included in routine examination of stool for ova and parasites (Weber & Rutala, 2002). Various different tests have been developed for the diagnosis of *Cryptosporidium* most of which involve direct detection by microscopic examination of tissues or fecal material using staining techniques. Diagnosis of cryptosporidiosis in humans and animals has evolved over time from histological staining to simple and more sensitive assays that are designed to detect oocysts or other antigens in stools samples (Ahmed & Karanis, 2018).

2.5.1 Staining methods for Microscopical Oocyst Detection

Presence of *Cryptosporidium* spp. oocysts in feces samples can be detected using the modified Ziehl-Neelsen staining technique as described by Clarke and McIntyre. Briefly, fecal smears are prepared on a microscope slide, air dried at room temperature, then fixed with absolute alcohol (methanol) for 5 minutes. The fixed smears are then stained with dilute carbol fuchsin (1: 10) for 3–5 minutes and washed with tap water. Smears are then decolorized using 3% acid alcohol (3% HCL in ethanol) for 10–15 minutes then counterstained with 0.5% malachite green solution for one minute. Smear slides are then washed with tap water, air dried, and

then examined under the microscope at x400 magnification (Clarke & McIntyre, 2001).

Cryptosporidium spp. oocysts will appear as pink to red, spherical to ovoid bodies against a green to purple background. Samples will be considered positive if at least one morphologically distinct *Cryptosporidium* spp. oocyst is observed (Koonakosit, Sriurairatna, & Petchclai, 1992). They appear as depicted in Figure 2.2 below.

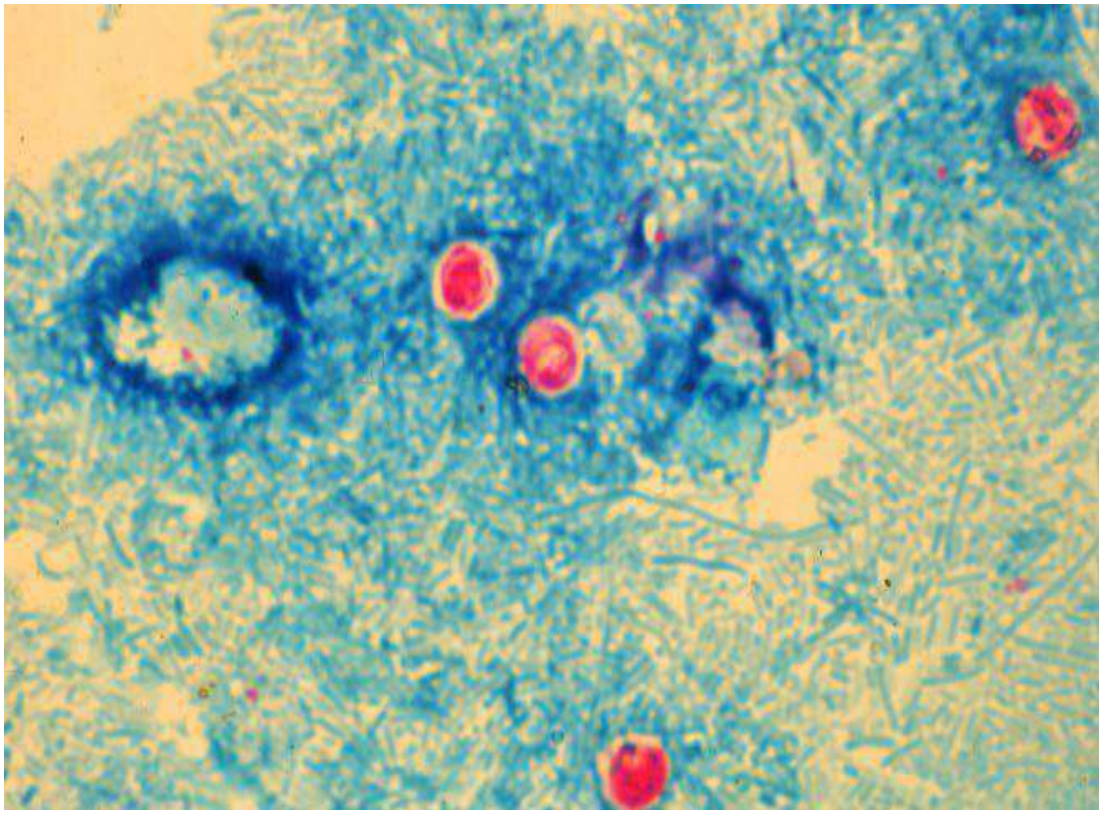

Figure 2.2: Cryptosporidium parvum oocysts appearing as bright pink to red organisms (Modified acid fast stain) (Chichino et al., 1991)

2.5.2 Detection of Cryptosporidium antigens by enzyme linked immunosorbent assay

In the ELISA, the presence of Cryptosporidium antigens in faeces (coproantigen) is sought. Depending on the commercial kit, Cryptosporidium coproantigens are captured and developed using a mixture of monoclonal and polyclonal antibodies. With the exception of increased throughput and automation, coproantigen detection kits do not offer increased sensitivity beyond the methods described.

Commercially available sandwich ELISA antigen detection kits contain anti-Cryptosporidium-coated well strips for capturing Cryptosporidium coproantigens, anti-Cryptosporidium antibodies for developing the reaction that is conjugated to an enzyme (frequently horseradish peroxidase), substrate, chromogen/ substrate development system and stopping solution (which inhibits further enzyme catalysis when added to the reaction mixture). These have been developed to detect C. parvum antigens in stool samples, but they may also be capable of detecting common epitopes from non-C. parvum infections. Known negative and positive samples are included in commercial kits. Commercial kits normally contain all the necessary reagents to perform the analysis and the manufacturers' instructions must be followed. It is false economy to dilute kit reagents to increase testing capacity. A comprehensive method and a formula for calculating the cut-off value and assigning positive or negative status to samples are usually included. Kit reagents are normally stored at 4°C when not in use. All reagents should reach room temperature before being used. The diagnostician should always determine whether any contraindications apply to the use of a commercial test and any stool/sample fixative used (Elgun & Koltas, 2011).

2.6 Clinical signs of Cryptosporidiosis

2.6. 1 Signs of Cryptosporidiosis in Cattle

Persistent discharge of yellow, watery feces containing mucus is suggestive of cryptosporidiosis. The most prominent signs of cryptosporidiosis are seen in pre-

weaned calves and include diarrhea accompanied by lethargy, inappetence, fever, dehydration and/or poor conditions. Reduced feed intake, varying degrees of apathy and dehydration maybe present. Only rarely do severe dehydration, weakness and collapse occur as in other causes of acute diarrhea in neonatal calves. The persistent nature of the diarrhea leads to a marked energy deficit and the calves die of inanition at three-four weeks of life (Santín, 2013). In the experimental disease in calves, depression and anorexia are the earliest and most consistent clinical findings. Feed intake is reduced and combined with persistent diarrhea over several days may cause emaciation (R. Fayer, Speer, & Dubley, 1990). However, recovery may occur between six and ten days after the onset of diarrhea. Both the incubation period and the clinical course of the diarrhea in calves affected with cryptosporidiosis tend to be a few days longer than diarrhea caused by rotavirus, corona virus or enterotoxigenic *E. coli* (Santín, 2013).

2.6.2 Signs of Cryptosporidiosis in Children

Cryptosporidium infection was first recognized in humans because of its association with severe diarrhea. The various symptoms differ greatly between immunocompetent and immunocompromised individuals. Diarrhea is the most noteworthy symptom. Cryptosporidiosis in humans typically manifests itself as a self-limiting disease with a median duration of 9–15 days, resulting in total recovery in healthy individuals. The major symptom is watery diarrhea associated with abdominal cramps, anorexia, weight loss, nausea, vomiting, fatigue and low-grade fever (Mor & Tzipori, 2008). Characteristically, the diarrhea is voluminous and watery, and often called cholera-like. However less fulminant diarrhea also occurs, even in HIV-infected persons (*Ghoshal et al*., 2018). Symptoms are similar in children and adults, although cryptosporidiosis acquired during infancy may have permanent effects on growth and development (Khalil *et al*., 2018).

Mucus may be associated with diarrhea, but blood or leucocytes are rarely reported. As much as 25-Kg weight loss have been reported. Other clinical symptoms besides diarrhea associated with cryptosporidiosis include cholecystitis, hepatitis, pancreatitis, reactive arthritis, and a variety of respiratory symptoms.

Cryptosporidium infection of the respiratory tract particularly in immunologically impaired patients has been the subject of a growing number of case reports (Mercado, Buck, Manque, & Ozaki, 2007). While *Cryptosporidium baileyi* infecting poultry produces respiratory symptoms in poultry it has been shown to cause infection in humans, exhibiting non-specific signs such as; shortness of breath, hoarseness, wheezing, croup and most often cough (Sponseller, Griffiths, & Tzipori, 2014).

2.7 Differential Diagnosis

These are diseases and conditions which have clinical signs or show clinical picture which is similar to cryptosporidiosis. Important differential diagnosis of cryptosporidiosis includes:

1. Other protozoa: *Giardia, Isospora, Microsporidia, Cyclospora, Eimeria, Toxoplasma.*
2. Enteric bacteria: *Clostridium dificile, Salmonella species, Shigella species, Campylobacter species, Mycobacterium avium complex.*
3. Viruses: *Cytomegalovirus, Adenovirus, Rotavirus.*
4. Adverse reactions to drugs: didanosine, clavithromycin, vitonavir.
5. HIV enteropathy.

2.8 Treatment of *Cryptosporidium* Infection

2.8.1 Treatment of Cryptosporidium in Humans

In immunocompetent individuals, general supportive care is the only treatment for the illness. Oral or intravenous rehydration and replacement of electrolytes is the single most important treatment to diminish clinical signs of disease in humans showing voluminous, watery diarrhea. Oral rehydration include: Gatorade, bonillon or oral rehydration solution containing glucose, sodium bicarbonate and potassium.

In immunocompromised hosts, particularly AIDS patients with CD4 cell counts below 200/mm3, cryptosporidiosis can be life-threatening and must be treated

aggressively. Initially, the nutritional, hydration, and electrolyte status of the patient should be assessed and corrected with intravenous hydration, if necessary. Anti motility agents, such as opiates and somatostatin analogues, may also be used. In such immunocompromised patients, the ideal treatment involves partial restoration of immune function with highly active antiretroviral therapy (HAART). Recently, the US Food and Drug Administration approved the drug Nitazoxanide (Alinia™) for the treatment of pediatric diarrhea caused by *C. parvum* and *Giardia lamblia* in children 1–11 years of age (Rossignol, 2010).

2.8.2 Treatment of Cryptosporidium in Animals

Animals suffering from cryptosporidiosis may require oral or parenteral rehydration with fluids and electrolytes in addition to anti-diarrheal and attempted chemotherapy with putative anti-cryptosporidial drugs. In bovine cryptosporidiosis, no specific anti-cryptosporidial drug has been identified. A few anti-coccidial or anti-protozoal drugs have demonstrable action upon the parasite. Claims for the efficacy of halofuginone lactate in calf cryptosporidiosis have been made by Swedish scientists (De Waele, Speybroeck, Berkvens, Mulcahy, & Murphy, 2010).

Most studies evaluating potential anti-cryptosporidial agents have been conducted in laboratory rodents. From the studies, many compounds such as maduramicin, alborixin, lasalocid, several aromatic amidines, salimomycin, dehydroepiandosterone, paromomycin, L - arginine, glucanthine, clarithomycin, azithromycin, erythromycin, oleandomycin, spiramycin, halofuginone, metronidazole, sulphadimethoxine, sulfamerazine, sulfamethazine, sulfaquinoxaline, mepacrine, norfloxacin and mefloquine show promise in laboratory rodents (Rasmusssen, Healey, Cheng, & Yang, 1995).

2.9 Prevention and Control of *Cryptosporidium* Infection

Cryptosporidiosis is difficult to control. Reducing the number of oocysts ingested may reduce the severity of infection and allow immunity to develop. *Cryptosporidium* oocysts can survive in chlorine-treated water body since it is

chlorine tolerant. This poses challenges for traditional chemical treatment of drinking and recreational water and for environmental surface cleaning. The organism also is not easily inactivated by alcohol-based hand sanitizers.

Prevention and control measures include the following (Yoder, 2012):

- practicing good hygiene (e.g., washing hands and not swimming when ill with diarrhea)
- Treating or avoiding contaminated water (not swallowing pool water, boiling or filtering water, and installing secondary disinfection systems (e.g., ultraviolet irradiation or ozone disinfection systems that inactivate *Cryptosporidium*) in pools
- Exercising caution when traveling by avoiding contaminated street food
- Avoiding fecal exposure during sexual activity
- Proper manure treatment before use as organic fertilizer on crops
- Routine surveillance on young calves to assess for level of prevalence
- Reducing contact between susceptible humans and infected animals
- Public education on the control of zoonotic diseases
- Prompt testing and treating calves showing diarrhea

CHAPTER THREE

MATERIALS AND METHODOLOGY

3.1 Study Area

This study was conducted in Asembo Location, Rarieda Sub County in Siaya County of Western Kenya. The Sub County lies approximately 57 Km to the West of Kisumu and borders Bondo Sub County to the north; Kisumu West Sub County to the East; and Homa-Bay and Suba Sub Countys to the Southeast and the South respectively across the Winam gulf. The study households were a subset of those enrolled in the Population Based Infectious Disease Surveillance (PBIDs) study (protocol number - KEMRI SSC No. 1899, CDC IRB No. 4566) in Asembo (Thumbi *et al.*, 2015). The PBIDs study conducts surveillance of human syndromes (fever, jaundice, cough and pneumonia) in 6000 households in 33 villages in the Asembo area. Ten out of these 33 villages were initially selected for the purpose of this study. We later moved into the other villages in order to achieve the required sample size. Most households (80%) in the study area were dependent on agriculture for their livelihood, and mainly practiced mixed crop-livestock production system. Majority of the households keep at least one species of livestock with chickens being the most common species (88%), followed by cattle (55%), goats (41%) and sheep (19%). Most households practice mixed small-scale farming with a median of 9 ruminants (cattle, goats and/or sheep) and 11 adult chickens (Thumbi *et al.*, 2015)

3.2 Study Design

We conducted a cross-sectional study. This study design was appropriate for determining the prevalence of *Cryptosporidium* Spp. in the selected calves as well as in the heaped manure. This design was also well suited to aid in measuring associations between *Cryptosporidium* positivity and associated factors for infection such as age of the calves, hygienic status, sex, source of water among other variables. Similar studies adopted the same study design in Tanzania and Ethiopia. (Swai & Schoonman, 2010).

3.3 Study Population

The study population consisted of calves aged 6 months and below and only one calf was selected from each recruited household. Samples from heaped manure were collected in half of the selected households with eligible calves.

For the questionnaire respondents, the household heads in the selected households were selected and interviewed.

3.4 Inclusion Criteria:

3.4.1 Inclusion criteria for calves

Calves aged 6 months and below in the selected households were eligible for recruitment into the study. In households with more than one eligible calves, the youngest one was selected and recruited into the study – only one calf per household was sampled.

3.4.2 Inclusion criteria for manure sample

Households with eligible calves and visible heaped manure in their farms were eligible for environmental sample collection for inclusion into the study. Heaped manure in this study meant collection of cattle manure, after cleaning the sleeping area, into a heap for further use either as fertilizer, fuel or building. Half the households would be sufficient to determine the environmental prevalence.

3.5 Variables

The following were the study variables:

3.5.1 Dependent Variables

The dependent variables were: Cryptosporidium positive in calf feces and environmental manure and Cryptosporidium negative in calf feces and environmental manure

3.5.2 Independent Variables

The independent variables were: age of calves, herd size, presence of loose stool, level of hygiene, type of calf feeding, source of water for calves, type of calf housing and frequency of manure removal from calves sleeping area.

3.6 Exclusion Criteria

3.6.1 Exclusion criteria for calves

Cattle older than six months were excluded from the study and so were households whose heads refused consent to participate in the study. Animals on anti-protozoal drugs were also excluded from the study. This information was obtained from the household head. Calves with obvious physical deformities e.g imperforate ani were also excluded from the study.

3.7 Sample Size Determination

3.7.1 Sample size determination for calves

The sample size was calculated using Cochran's (1977) formula as follows; (Cochran, 1977)

$$n = \frac{z^2 . P_{exp} (1 - P_{exp})}{d^2}$$

Where,

n=sample size

Z=Z statistic for a level of confidence (95%)

P_{exp}=expected prevalence (35%) (Swai & Schoonman, 2010)

d=precision (5%)

$n=1.96^2*0.35*0.65/0.05^2$

n=349.5

n = 350

3.7.2 Environmental manure samples

Samples from visible manure in the environment (household compound) were collected from 50% of all the households with eligible calves. These were randomly selected by sampling every second pile of manure encountered within the household's compound or calf shed.

3.7.3 Sampling Procedure

A list of all households that owned calves aged six months and below in each of Asembo's locations, was obtained from an ongoing population-based animal syndromic surveillance study in the area (Thumbi *et al*., 2015). This formed the sampling frame as shown in page 66.

From the list of households with eligible calves, 385 herds, each with at least one eligible calf, were selected randomly using simple random sampling procedure with the aid of computer-generated Random numbers generator. Since each household had a unique identification number, 385 households' unique identification numbers were randomly generated. The calves in these households formed the study population. In households with more than one calf aged six months and below, the youngest calf was picked.

The owners of the selected households were invited to participate in the study through a letter which included a brief description of the study objectives.

3.8 Fecal sample collection

3.8.1 Collection from calves

During the visit to each household herd, a fresh rectal fecal sample was collected from the selected calves into a sterile, airtight, 10mL plastic falcon tube. Fecal collection was done ethically in compliance with animal welfare by trained animal health practitioners and did not cause distress to the animals. The fecal samples were labelled and transported in a cool box to the KEMRI laboratories in Asembo and later to Kisian within 6 hours of collection.

3.8.2 Environmental manure

In every second household where there was visible manure within the compound or in the animal sleeping area, a sample, approximately 50g, of thoroughly mixed manure was collected and placed in a sterile airtight 50ml plastic tube. The samples were labeled with the household's identification and transported the same day to the KEMRI laboratory at Asembo then later to Kisian for processing.

3.9 Laboratory Detection of *Cryptosporidium* in Feacal and Environmental manure samples

Presence of *Cryptosporidium* spp. oocysts in the fecal samples was detected using the modified Ziehl-Neelsen staining technique as described by Clarke and McIntyre (Clarke & McIntyre, 2001). Briefly, fecal smears were prepared on a microscope slide, air dried at room temperature, then fixed with absolute alcohol (methanol) for 5 minutes. The fixed smears were stained with ZN Carbol fuchsin (1: 10) for 3–5 minutes and washed with tap water. The stained smears were then decolorized using 3% acid alcohol (3% HCL in ethanol) for 10–15 minutes then counterstained with 0.5% malachite green solution for one minute. Smear slides were then washed with tap water, air dried, and then examined under the microscope at X100 magnification.

Cryptosporidium spp. oocysts appeared as pink to red, spherical to ovoid bodies against a green to purple background. Samples were considered positive if at least one morphologically distinct *Cryptosporidium* spp. oocyst was observed.

This staining method was used because of its high sensitivity and specificity, simple and affordable to perform. It also aids in distinguishing *Cryptosporidium* oocysts from yeast which are about the same size and shape as the *Cryptosporidium* oocysts. The yeast will stain with the green counterstain.

3.10 Data Management and Analysis

3.10.1 Data collection

Data was collected by use of a pre-tested structured questionnaire see 0In as much as the study population in this study were the calves, information about consent and associated factors was collected from the calves' owners as is the case in most animal studies. The questionnaire was designed to comprise mostly closed ended (categorical) questions to ease data processing, minimize variation and improve precision of responses. The questionnaire collected information about the potential risk factors for cryptosporidium which included age of the calf, consistency of feces (whether normal or diarrheic), level of hygiene (which was assessed by observation and categorised using a tool), herd size, manure handling and uses, sources of water, season (whether wet or dry) and possibility of infection by animal manure, general knowledge about the disease and household practices that might lead to human exposure to zoonotic diseases. To assess knowledge of the disease by calf owners, the clinical picture of the diarrhea due to Cryptosporidiosis (yellowish, watery and containing mucus) was described to the interviewee in their local language. Hygiene level was estimated based on the frequency of manure removal, frequency of cleaning of the animal's sleeping area and presence of visible slurry on the floor. From these factors we developed a scoring system with two categories i.e Good/Moderate and poor. Places that were cleaned daily and appeared dry with very little or no observable slurry were considered good/moderate hygiene level whereas those which appeared to be generally wet, dirty and with slurry were categorized as

poor hygiene level. Frequency with which children, being at higher risk of infection, came into contact with the animals was also assessed.

3.10.2 Data analysis

Data from questionnaires on calf level, herd-level factors and human factors was entered, cleaned and analyzed using EPI Info 7 (CDC, Atlanta, GA, USA) and Ms Excel 2007 (Microsoft, Seattle, WA, USA). Frequencies and proportions were calculated for the categorical variables and measures of central tendency and dispersion for the continuous variables.

We calculated odds ratio (OR) and 95% confidence interval (CI) for associations between the presence of *Cryptosporidium* oocysts and potential factors. We performed logistic regression to examine independent factors, for which factors with p-value _< 0.15 from univariable analysis were included into the multivariate logistic regression model and adjusted odds ratios (AOR) and 95% CI calculated. We used a forward step-wise selection method. Factors with p-value _< 0.05 were retained in the final model after exploring all statistically and biologically plausible interactions among the variables remaining in the final model.

3.11 Ethical Approvals and Considerations

Protocol approval was sought and obtained from Board of post graduate studies of Jomo Kenyatta University of Agriculture and Technology (JKUAT) and ethical clearance was sought and obtained from The Jaramogi Oginga Odinga Teaching and Referral Hospital Ethics & Research Committee (JOTRH-ERC) in Kisumu, Kenya referenced ERC.1B/VOL1/167 (0)

The aim and procedures of the study was explained to calf owners who were required to give consent prior to recruitment of their calves into the study. Fecal samples were collected from the selected calves belonging to consenting owners. In case an owner of selected herd declined to consent, another eligible household was randomly selected to replace it. The collected fecal samples were only used to detect *Cryptosporidium* oocysts. Confidentiality of laboratory information was observed

and maintained by use of password protected database and the questionnaires were kept under lock and key.

CHAPTER FOUR

RESULTS

4.1 Distribution of Calves Characteristics

Fecal samples were collected from 350 calves aged 6 months and below while 187 environmental manure samples were collected from 187 (53%) households. The age distribution is as depicted in Figure 4.1. The mean age for the calves was 4.3 months.

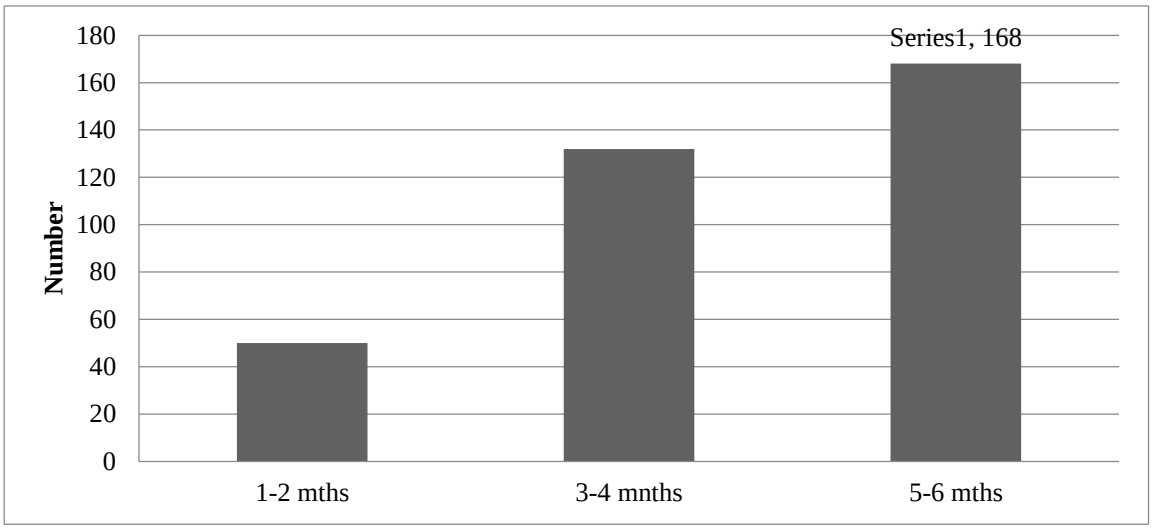

Figure 4.1: Age distribution of the studied calves

Most calves, 85% (297/350) were from herds with less than 10 animals. The smallest herd had three animals while the largest had 19 animals. Among all the sampled calves, 15%, 51/350 had visible loose stool at the time of sampling and in order to assess whether the calves had contracted the diarrhea from previously diarrheic animals in the herd, we asked whether any other animal had presented with diarrhea over the last three months preceding calf sampling. Of the 350 herds, 33% (116/350) reported to have had at least one animal having showed signs of diarrhea within three months preceding the sampling. These and other characteristics are shown in Table .

Table 4.1: Distribution of Calves' Characteristics

Characteristic	Level	Frequency N=350	Percentage (%)
Age			
	1-3 months	118	34
	4-6 months	232	66
Location			
	East Asembo	86	25
	Central Asembo	85	24
	West Asembo	91	26
	South Asembo	88	25
Herd size			
	1-10	297	85
	11-20≥11	53	15
Calf Diarrheic			
	Yes	51	15
	No	299	81
Feces on coat			
	Yes	47	14
	No	303	87
Sampled calf ever had diarrhea in last 3 months			
	Yes	62	18
	No	288	83
Any animal in herd had diarrhea in last 3 months			
	Yes	116	33
	No	234	67

In assessing calf management practices, factors such as calf housing, level of hygiene, calf feeding and watering were assessed among other factors. In terms of calf housing, 112 (32%) of the calves were housed in the household kitchens whereas 103 (29%) had calf pens. Only 29% of the households interviewed were aware that cryptosporidiosis is zoonotic.

Further descriptions of the studied factors are as shown Table .2 below;

Table 4.2: Calf Management Practices in the Study Households

Category	Levels	Frequency (n)	Frequency (%)
Do you keep other livestock	No	1	0.3
	Yes	349	99.7
Level of Hygiene	Good	69	19.7
	Moderate	187	53.4
	Poor	94	26.9
Calf feeding	Free-grazing	74	21.2
	Stall-feeding	6	1.7
	Tethering	269	77.1
Calf watering	Animals go to water	96	27.4
	Water provided at household	254	72.6
Source of water for calf	Tapwater	41	11.7
	Rain	59	16.9
	River/lake	78	22.3
	Ponds	192	54.9
Calf Housing	Calf pens	103	29.4
	Kitchen	112	32.0
	Open	54	15.4
	Shed	81	23.1
Disinfect calf sleeping area	No	309	88.3
	Yes	41	11.7
Accessed vet services last 3 months	No	147	42.0
	Yes	203	58.0
Presence of feces on animal coat	No	302	86.5
	Yes	48	13.5

4.2 Prevalence of *Cryptosporidium* Spp. Infection

We found a Cryptosporidium species prevalence of 8.3% (95% CI 5.7 – 11.53) among calves and 7.5% (95% CI 4.3 – 11.9) in the environmental samples.

Calf ages were categorized in two groups as 1-3months and 4-6 months and these recorded a prevalence 96% (n=118) and 4% (n=232) respectively.

4.3 Relationship between the Investigated Risk Factors and *Cryptosporidium* Infection

At univariable analysis, calves showing signs of diarrhea were associated with higher risk of infection with cryptosporidium spp. (Odds ratio [OR] =11.9, 95% C.I 5.3-21.1) for diarrheic calves compared to normal stool consistency. Younger calves aged 2 months and below also showed a higher risk of infection (OR=12.3, 95% C.I: 5.4 – 28.1) compared to the older ones. Prevalence of *Cryptosporidium* infection in calves was significantly higher during the wet season compared to the dry season (OR=2.2, 95% C.I 1.1-4.7). For the environmental samples, there was no association between the dry and the wet season since none was positive during the dry season (OR=0, p=0.001). The common practice of heaping manure showed no association with *Cryptosporidium* infection same as sex of the calves. Calves from households with positive manure samples were at higher risk of being cryptosporidium positive (O.R=9.8, 95% CI: 3.1–31.5) compared to calves from households with negative manure samples. In fact, 12 (85%) of the households where manure samples tested positive for presence of *Cryptosporidium* oocysts also had calves that were positive. Calves raised in poor/dirty environments (O.R=17.2, 95% CI: 6.3-46.8) had higher chances of *Cryptosporidium* infection compared to those raised in good/moderate hygiene conditions. Feeding of supplements to the calves appeared to be protective though not significant (OR= 0.36, 95% C.I 0.12-1.07, p=0.08). Larger herd sizes did not affect the level of prevalence of *Cryptosporidium* infection in both calves and the environment with (OR=2.33, 95%CI 0.98-5.59) and (OR=3.14, 0.98-10.12) respectively.

Multivariate analysis identified that calves showing signs of diarrhea (AOR=6.1, 95% CI 2.2 – 17.0), calves raised in poor hygiene conditions (AOR=10.0, 95% CI 3.3 – 30.9) and calves aged two months and less (AOR=12.9, 95% CI 4.6 – 35.8) were significantly associated with Cryptosporidium positivity while adjusting for these factors simultaneously as shown in Table **.**

Table 4.3: Relationship between risk factors and cryptosporidium infection

Factor	Levels	Crypto positive (n)	%	Crypto negative (n)	%	OR (95% CI)	AOR (95% CI)
Age of calf	≤2 months	17	59	33	10	12.3 (5.4-28.1)	12.9 (4.6-35.8)
	>2 months	12	41	288	90	Reference	
Herd size	11-20 calves	8	28	45	14	2.3 (1.0-5.6)	NS
	1-10 calves	21	72	276	86	Reference	
Loose stool	Present	17	59	34	11	11.9 (5.3-27.1)	6.1 (2.2-17.0)
	Absent	12	41	287	89	Reference	
Presence of other diarrheic animals within herd	Present	19	66	97	30	4.4 (1.2-9.8)	NS
	Absent	10	34	224	70	Reference	
Sex of calf	Female	19	66	163	51	1.8 (0.8-4.1)	
	Male	10	34	158	49	Reference	
Presence of surface Run-off in the compound	Present	17	59	131	41	2.1 (0.9-4.4)	NS
	Absent	12	41	190	59	Reference	
Feeding of calf on commercial supplement	Yes	4	14	98	31	0.3 (0.1-1.1)	NS
	No	25	86	223	69	Reference	
Calf watered from Pond	Yes	24	83	168	52	4.3 (1.6-11.7)	NS
	No	5	17	153	48	Reference	
Calf Housing type	Other places	15 (52)		223 (69)		0.5 (0.2-1.0)	NS
	Kitchen	14 (48)		98 (31)		Reference	
Hygiene Level	Poor	24		70		17.2 (6.3-46.8)	10.0 (3.3-30.9)
	Good/Moderate	5		251		Reference	

4.4 Manure Handling Practices Among The calf owners

In Asembo, 78% (274) of the studied households use animal manure as fertilizer on their crops while 22% (75) use it for building purposes (use on walls and floors of mud houses). Only 20% (68) of the households did not consider washing their hands after handling animal manure as important whereas the remaining 80% always washed their hands after handling manure. With regards to frequency of manure removal from the animals sleeping area, majority 55% (190) removed on need basis while only 7% (23) remove manure from animal sleeping area daily. Further details regarding manure handling are as depicted in Table below.

Table 4.4: Distribution of Manure Handling practices Among Calf Owners

Category	Level	Frequency	%
Heaping manure	No	83	23.71%
	Yes	267	76.29%
Frequency of manure removal	Daily	23	6.67%
	Monthly	43	12.46%
	Never	21	6.09%
	On need basis	190	55.07%
	Weekly	68	19.71%
Uses of manure	Crops	274	78.29%
	Building	75	21.43%
	Other	1	
Hand washing after handling manure	No	68	20.00%
	Yes	272	80.00%
Presence of run off	No	202	57.71%
	Yes	148	42.29%

4.5 Zoonotic Risk of Cryptosporidium

In order to assess the zoonotic risk of infection posed by the calves to the people living close to them, the calves' owners were asked about their interactions with the calves using the questionnaire see on page 54. Out of the 350 calves owners, 70% (246/350) were not aware of any disease that they could acquire from getting into contact with animal feces/manure. Of the 350 households, 112 (32%) did not have children under the age of five years while the remaining 68% (238) had children aged below five years. We assessed the frequency with which these children, being at higher risk of zoonotic infection, come to contact with the animals and found out that 50% (120/238) of them get into contact frequently while 26% (61/238) of them never come into contact with the animals. We also wanted to know whether animals are restricted from accessing the water sources used by the families and found out that 83% (293/350) of the households have their water sources not restricted from animal access. When asked whether they thought *Cryptosporidium* organisms could contaminate milk, 44% (154/350) said no while the remaining 56% (196/350) said it was possible for *Cryptosporidium* organisms to contaminate milk. These and more details on the risk of zoonotic transmission of cryptosporidiosis are highlighted in table 4.5.

Table Table 4.5: Zoonotic Risk of Cryptosporidium Infection Among Calf Owners

Category	Level	Frequency	%	95% CI
Can one contract zoonotic disease from animal feces	No	246	70.29%	65.15-74.97%
	Yes	104	29.71%	25.03-34.85%
Children under Five	No	112	32.00%	27.20-37.21%
	Yes	238	68.00%	62.79-72.80%
Frequency of contact	Frequently	120	50.21%	43.6956.72%

	Never	61	25.52%	20.12-31.54%
	Occasionally	58	24.27%	18.97-30.21%
Animals graze near food crops	No	36	10.29%	7.4014.07%
	Yes	314	89.71%	85.9392.60%
Water restricted from animal access	No	293	83.71%	79.33-87.34%
	Yes	57	16.29%	12.6620.67%
Can feces contaminate milk	No	154	44.00%	38.75-49.38%
	Yes	196	56.00%	50.6261.25%

4.6 Disease knowledge and perception Among Calf Owners

Majority of the calves owners interviewed responded that cryptosporidiosis is a preventable disease with 118 (33%) saying it can be prevented through deworming. On whether the disease is seasonal or not, 66% (233/350) thought it was seasonal, 23% (80) thought it was not while the remaining 10% (36/350) said they didn't know. More details on disease knowledge and perception are in Table 4.6 below.

Table 4.6: Disease knowledge and perception Among Calf Owners

Category	Level	Frequency	%
Is it preventable	Yes	210	60.00%
	No	140	40.00%
Prevention modes	Hygiene	48	13.71%
	Deworming	118	33.71%
	Own Treatment	82	23.43%
Is it seasonal	Don't Know	36	10.32%
	No	80	22.92%
	Yes	233	66.76%
Does it affect one or group	Group	249	71.35%

	One	100	28.65%
Ages affected	Adults	66	18.91%
	All Ages	170	48.71%
	Calves	113	32.38%

CHAPTER FIVE

DISCUSSION, CONCLUSIONS AND RECOMMENDATION

5.1 Discussion

In this present study, we report prevalence of *Cryptosporidium* infection in calves and the environment in an area where a previous study had reported high *Cryptosporidium* infection in children presenting with diarrhea (Kotloff *et al*., 2013) . Other studies in Malindi, Dagoretti and Tanzania reported prevalence of 10.6%, 7.7% and 35% in calves respectively (Ali, 2010; Kang'ethe *et al*., 2012; Swai & Schoonman, 2010). Further, we identified factors associated with greater risk of *Cryptosporidium* positivity in calves and their implications in disease transmission and control.

Higher rates of infection were reported during wetter season of the year. This shows that *Cryptosporidium* infection could be a seasonal disease whose prevalence is mainly affected by precipitation. Most of the calf owners interviewed also responded that there is a seasonal occurrence of diarrhea similar to Cryptosporidiosis in the calves, with higher incidences during the rainy season compared to the drier seasons. This is consistent with many other studies in Kenya, Rwanda and Zambia (Lepage, D, & J, 1987; Muchiri *et al*., 2009; Siwila, Phiri, Enemark, Nchito, & Olsen, 2011).

Diarrhea in calves was significantly associated with Presence of *Cryptosporidium* oocysts showing that it is a major cause of morbidity and mortality due to diarrhea. This finding is also evident in other studies carried out in Ontario, Canada (Trotz-Williams *et al*., 2007). However, in Tanzania, a higher prevalence of 35% was reported in apparently healthy calves. This explains the fact that *Cryptosporidium* infection exists both symptomatically and asymptomatically (Swai & Schoonman, 2010). A study by Cheekley on children showed that presence of *Cryptosporidium* infection affects weight gain in both asymptomatic and symptomatic children, with the latter gaining much less weight (Checkley *et al*., 1997).

The study found that younger calves below 2 months had greater chances of getting infected with *Cryptosporidium* compared to older calves. This finding is consistent with other studies which reported similar correlation with age of calves (Swai & Schoonman, 2010; Trotz-Williams *et al.,* 2007). Young infected calves play an important role in maintaining infection both in the herd and environmental contamination thus representing the greatest zoonotic risk to humans.

On disease awareness, 70% of the interviewed people were unaware of any disease that they could acquire from handling cattle manure and 44% do not know that *Cryptosporidium* oocysts can contaminate milk. This shows that public education is needed in order to correct this misconception in order to mitigate its impact in humans (Vermeulen *et al.,* 2017).

Sharing of same water sources between humans and animals is a common practice in Asembo and this increases chances of zoonotic transmission of *Cryptosporidium* Spp. to humans. On surface waters, high levels of contamination with *Cryptosporidium* parvum and hominis was reported in a study conducted in Eastern part of Kenya (Muchiri *et al*., 2009). *Cryptosporidium* Spp. has also been reported to cause several waterborne outbreaks in humans in developed countries, with the most notable one being in the USA and UK.

Presence of children aged below 5 years and their frequent contact with cattle in Asembo increases the risk of zoonotic transmission since children are more vulnerable to *Cryptosporidium* Spp infection than adults as shown in various other studies (Khalil *et al.,* 2018; Kotloff *et al*., 2013). Their contact with cattle, both infected and uninfected, should therefore be reduced so as to decrease the risk of zoonotic transmission.

The practice of housing calves in the kitchens used by people for cooking increases closer contact between calves and humans thereby increasing the risk of cross-infection by *Cryptosporidium* Spp. (Trotz-Williams et al., 2007).

Our study showed presence of *Cryptosporidium* oocysts in heaped manure. Most of the people interviewed collected manure into heaps and used it as fertilizer in their food crops in its raw form without any form of treatment. This poses a serious health hazard as the crops may easily become contaminated with the *Cryptosporidium* oocysts which survive for long in the environment thereby increasing risk of transmission to humans through eating contaminated food. The warm and humid climate in Asembo could aid in *Cryptosporidium* Spp. persistence and spread in the environment. Farmyard manure may thus contain high numbers of *Cryptosporidium* oocysts and, consequently, water may be contaminated by the manure or slurry washed into rivers and vegetable crops may also be contaminated by direct manuring of the fields in which they are grown. Other studies have shown that contaminated manures from dairy or beef cattle operations can be major sources of *Cryptosporidium* oocysts unless manure management or treatment strategies are used to minimize oocysts viability or transport to water (Vermeulen *et al*., 2017).

Since there was near similar prevalence of *Cryptosporidium* oocysts in the calves and the environment and it is easier to sample the latter, environmental sampling may be just as effective as animal sampling in future studies. This is due to the fact that it is more cost effective and causes no stress to the animals. The prevalence reported in such studies can be used as proxy indicators of the prevalence in animals in resource-poor settings.

Few people remove cattle feces from the animal sleeping areas on a daily basis. To worsen the situation, only 11% disinfect the animal sleeping areas. This has an effect of ensuring persistence of Cryptosporidium oocysts in the environment.

About a third of the calf owners interviewed thought that this infection could be prevented by deworming the calves. This shows the misconception of this organism. Most people think it is a worm while it is a protozoal organism.

Presence of a diarrheic animal in the herd increased the risk of *Cryptosporidium* infection in the calves showing a possibility of transmission from other sick animals.

Contrary to other studies in the United States, we did not find any significant association between larger herd sizes and increased infection rates (Garber, Sahman, & Hurd, 1994). Although majority of farmers in our study area practiced small-scale livestock keeping.

The risk factors significantly associated with Cryptosporidium infection in calve in this study were; age (<3months), presence of diarrheic animal in the herd, poor hygiene, calf being diarrheic, drinking water from pond and wet season of the year. Our significant risk factors are consistent to other factors found to be significant in other studies conducted under similar conditions (Garber *et al.*, 1994; Swai & Schoonman, 2010; Trotz-Williams *et al.*, 2007).

The overall prevalence reported shows the presence of *Cryptosporidium* Spp. both in the calves and environment. The potential of human infectivity by the *Cryptosporidium* oocysts identified in our study could not be established since we used microscopy (mZN) as our tool of diagnosis. Whereas mZN is commonly used for testing presence of *Cryptosporidium* oocysts, it is worth noting that microscopy might also have under-estimated the actual burden and prevalence of *Cryptosporidium* infection in Asembo since mZN has lower sensitivity compared to PCR. The mZN is a widely used screening test for *Cryptosporidium*; however it is not specific enough to discern species as would a molecular sequencing test (Tahvildar-Biderouni & Salehi, 2014). Not all *Cryptosporidium* species are zoonotic (Xiao & Feng, 2008); therefore, we could not establish the proportion of zoonotic species among our positive cases, which would have enabled us to quantify the zoonotic risk posed by the infected calves. Further studies should include molecular diagnosis to identify the parasites to the species level and thus correctly quantify the zoonotic potential of this parasite. This knowledge will aid in packaging public health information on control strategies can then be targeted towards the population at risk of zoonotic infection thus curtailing the spread to humans since there is no effective medication against Cryptosporidiosis and prevention remains the best and plausible method of control. With the increasing population of immunocompromised

individuals, control of *Cryptosporidium* Spp. at the animal and environment level would help protect humans from infection.

5.2 Conclusion

From our findings, the following conclusions are made;

- The prevalence of *Cryptosporidium* Spp. in calves aged 6 months and below in Asembo, Siaya County was 8.3%.
- The detected presence of *Cryptosporidium* oocysts, at a prevalence of 7.5% in heaped manure indicates the high level of environmental contamination by infected animals. Improper handling of animal manure poses a risk of infection to both humans and animals. The risk of infection to humans and calves from the contaminated manure could further be increased due to the fact that the oocysts can persist for long periods in the environment and are resistant to most commonly used household disinfectants.
- Cryptosporidium positivity was higher among calves aged below two months and calves raised in poor hygiene conditions. Calves with loose stool also showed higher prevalence; however we could not establish whether the loose stool was due to *Cryptosporidium* infection. Infected animals are potential reservoirs for zoonotic *Cryptosporidium* which can infect humans living in close contact with them.
- Poor hygienic condition was associated with increased risk of *Cryptosporidium* positivity in calves and this calls for public education on the importance of maintaining high hygiene standards as a means of preventing diseases to both humans and livestock.

5.3.1 Recommendations

- Since there was a near equal prevalence of presence of *Cryptosporidium* oocysts in both the calves and the sampled environmental manure, we recommend that when resources are not sufficient to collect rectal feces from

calves then environmental sampling can be used as a proxy to estimate the prevalence in calves.

- Intervention strategies should be targeted towards young calves to reduce transmission within herds and into the environment, thereby curtailing the zoonotic pathway. This is because the young calves are more vulnerable to infection.
- Heaped manure for use as fertilizer or other uses should be treated before use to reduce chances of infection transmission to humans through contamination of water sources and food crops
- Since there are no effective medications against *Cryptosporidium* infections both in humans and calves, maintenance of high hygiene standards remains the surest way of controlling its spread.

5.3.2 Recommendation for further areas of study

We recommend molecular studies in order to identify the zoonotic species of *Cryptosporidium* Spp. since not all the species have zoonotic potential.

We also recommend longitudinal studies to determine the effects of *Cryptosporidium* infections in the growth of young calves, the impact on their future potential productivity and the impact of seasonality on *Cryptosporidium* infection.

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APPENDICES

Appendix I: Study Questionnaires (English version)

Animal questionnaire

Interview Date _____

Interviewer ID code _____

Household ID _____

Animal details

1. Animal ID _____
2. Visit number _____
3. Age in months _____
4. Is the animal diarrheic _____ (Yes/No)
5. In the last 3 months, has the study animal suffered from any diarrheal disease?_____(No/Yes)
6. In the last 3 months, has any other animal in the herd suffered from any diarrheal disease? ____(No/Yes)

Disease knowledge

7. Is there seasonality or other timing to the appearance of the diarrheal disease?
8. Does the disease affect one animal or a group of animals at the same time?
9. What ages of cattle are affected by the diarrheal disease?
10. Are there ways to prevent/avoid this disease? If so, what are they?

Animal husbandry and management

1. How many cattle do you own?_____
2. What other livestock do you keep?
3. What is the level of cleanliness of the farm? Researcher to observe and score as per the described criteria.
4. How do you feed your calves _____(free-grazing/stall-fed)
5. Do you feed your animals on any supplements _____(None/crop residues/minerals/concentrates)
6. How are your animals watered _____ (water provided at household/animals go to water)
7. What is the source of water that you use? _____ (Shallow well/River or Lake/Tap water/Rain water)
8. How do you house your calves? _____ (Calf pens/free range/housed with other animals)
9. Have you accessed veterinary services in the last 3 months? _____(Yes/No)
10. Are there any preventive treatments/Vaccinations on cattle carried out in the farm in the last 3 months_____(None\deworming/tick-control/vaccination/prophylactic treatment)
11. Do you disinfect the animal sleeping area? _____ (Yes/No)
12. If yes, which chemical do you use?
13. Are there feces on the coat/ tail of the calf?

Manure handling

14. Do you collect the animals feces/manure into one heap?_____ (Yes/No)
15. What do you use the manure for? (Building/Applied on crops/Other)
16. How often are feces removed from the shed?

    (Daily/ Weekly/ Monthly/ Never)

17. Do handlers wash hands after handling manure/ feces?
18. Is there any run-off from cattle shed? (Researcher to observe)

    (Yes/ No/ Cannot observe)
19. If yes, where does it go?

Zoonotic Risk

20. Are you aware of any disease you can get from handling cattle feces? (Yes/No)
21. Do cows have access to well verges/ drains or water fronts? (Yes/No)
22. Do you have children under the age of five years? (Yes/No)
23. How often do children under five years come into contact with the farm animals? (Frequently/ Occasionally/ Never)
24. Are wells:
    a. Covered? (Yes/No)
    b. Raised? (Yes/No)
25. Is it possible for dung to contaminate milk? (Yes/No)
26. Do cows graze near food crops? (Yes/No)
27. Is manure visible on farmyard? (Yes/No)

Appendix II: Criteria to be used to assess hygiene of the farm

Rating	Characteristics
Good	Separate calf pens, which are dry, spacious, no feces, cleaned daily, calves clean, clean floor
Moderate	Separate calf pens which are washed occasionally, minimal feces present on the floor and on the calves
Poor	Very dirty, floor not dry, feces present all over, calves dirty

Appendix III: Consent form (English version)

Title of study:

Prevalence and associated Factors for Cryptosporidium Infection in Bovine and Environment, Asembo: Rarieda Sub County

Introduction:

My name is Dr. Allan Fredrick Ogendo. I am trying to learn more about Cryptosporidiosis in animals and environment. Cryptosporidiosis is a zoonotic disease that is of public health importance. It can be transmitted from animals to humans when people get exposed to manure from the infected livestock.

Purpose of study:

Due to the increasing public health importance of this disease, I am requesting for your participation in this study whose main objective is to find out how many of your animals in this village and other villages in Rarieda Sub County are exposed to this disease, what are the factors associated with transmission or acquisition of this disease by your animals. This will be important for the relevant authorities to find ways of dealing with this disease in this area. You are being asked to join this study because your household was picked by chance among others in this area.

Expectations of the study:

If you agree to participate in the study, I wish to test one of your cattle aged below 6 months and a sample from you heaped manure to test if they could have been exposed to Cryptosporidiosis. If you agree to take part in the study, a trained animal health assistant will collect 50 grams of feces from your animal using sterile gloves and the same amount from your heaped manure. The feces will be transported to KEMRI Kisian laboratory where I will test for Cryptosporidiosis. I shall then ask you some questions which are written on a paper on animal husbandry and your practices regarding Cryptosporidiosis. The test results shall be availed as soon as possible to

Sub County veterinary officer and Sub County medical officer of Health of Rarieda who shall forward them to you and advice on any necessary control measures if need be.

Risks:

Handling and restraining animals for sample collection can be slightly stressful for the animals. Every care will be taken to minimize this stress. If you are asked to assist with animal restraint, there is some risk of injury. Participation with restraint is completely voluntary and nothing will be asked of you outside your normal husbandry practice with your animals. Collection of fecal samples will not cause pain to the animals. Sampling the animals may take some time, as will answering the questions about the animals.

Benefits:

The results of this study will enable us to know the prevalence of Cryptosporidiosis in this region. Recommendations from the study will include putting in place prevention and control measures against the disease.

Confidentiality:

Any information obtained from you will be kept confidential and used solely for purposes of this research only. The results of this research may be published in scientific journals or presented at medical or veterinary meetings, but your identity will not be disclosed.

Compensation:

If you accept to take part in this study, there will be no payment for participation.

Alternatives:

You have a choice to agree or not to agree to participate in this study. If you agree to participate in study you are allowed to withdraw from the study at any time if you so wish without any consequences whatsoever.

Consent:

The consent form has been explained to me and I agree for my household and animals to take part in the study. I have been told that I am free to choose not to take part in this study at any time and that saying "NO" will have no effect on my family or me and will not affect my participation in other studies.

Name of participant……

Household ID…….

Signature/ thumb print of participant……..

Date……..

Name of researcher/research assistant……..

Signature…….. Date …….

Appendix IV: Translated consent form (Dholuo version)

Title of study:

Prevalence and associated Factors for Cryptosporidium Infection in Bovine and Environment, Asembo: Rarieda Sub County

Motelo:

Iluonga ni Dr. Allan Fredrick Ogendo. Atimo nonro mar tuo ma iluongo ni Cryptosporidiosis (En tuo mar diep kendo diep no en ratong') kuom dhok kod owuoyo. Tuo ni dhano nyalo yude ko a kuom dhok to kendo onyalo mako oganda mang'eny. Dhano nyalo yude ka gimulo minyaga mar dhok ma ni kod tuo ni.

Purpose of study:

Nikech tuo ni nyalo mako oganda mang'eny, akwayo ni mondo iyie idonj e nonro ni. Wadwaro ng'iyo ni be kute mag tuo ni yudore e dhogi kata e owuoyo mar dhok e gweng'ni kod gwenge ma nitie e aluora u. Wanono timbe ma nyalo miyo tuo ni o medre kuom dhok. Dwoko ma wayudo biro konyo migepe mag sirkal mondo o los yore mag geng'o tuo ni kuom oganda. Wakwayi ni mondo I donj e nonro ni nikech odi ni ne o yier

Expectations of the study:

Ka iyie donjo e nonro ni, wakwayo ni mondo wapim dhiang' achiel ma nitiere e bwo dueche auchiel. Ka I yie to wabiro kawo minyaga matin maromo grams 50 kuom dhiang kod owuoyo. Minyaga ni ibiro or e lab mar KEMRI ma nitie kisian mondo o pim. Abiro penji penjo moko matin mar ng'eyo kaka udak gi jamni to kod weche mag ler. Dwoko mag nonro ni ibiro miu koluwo afis mar veterinary kod mar thieth.

Rach bedo e nonro:

Mako kendo tweyo jamni nyalo kelo midhiero matin ne jamni. Wabiro kawo okang' mondo waduok midhiero ni chien. Ka okwayi mondo ikony e geng'o jamni, inyalo

hinyori. Konyo e geng'o en heroni kendo onge kwayo moro maloyo arita mapile mag jambi. Golo minyaga ok bi kelo rem ne jamni. Golo minyaga nyalo kawo thuolo, kaa achiel kod dwoko penjo mag jamni.

Ber bedo e nonro:

Duoko mag nonro biro miyo wangeyo pek mar tuo ni e jamni mag aluora ni. Machielo en rieko ma biro medore kendo nyalo kelo konyruok kod thieth mar Cryptosporidiosis.

Maling' ling':

Duoko mag nonro ni ok bi yangi ne joma moko to ibiro mana ti kodgi e nonro ni kende. Duoko gi inyalo mana ndiki e oboke mag jo sayans kata inyalo wach gi e twak mag jo sayans.

Chudo:

Ka I yie donjo e nonro ni to onge chudo ma ibiro yudo.

Ratiro:

In kod ratiro mar yie donjo e nonro kata tamori donjo. Ka I yie donjo e nonro to bende o yie ni wuok e nonro saa a saya ma onge kum moro a mora ma ibiro yudo.

Yie:

Oselerna weche andikani mar yie to ayie ni jodalana gi jamni mondo odonj e nonroni. Onyisa ni an thuolo yiero ni kik adonj e nonroni sama adwaro kendo bende wacho ni ooyo ok bikelona kata jodalana kum moro a mora kata lokruok e yor donjo e nonro mabiro

Nyingi ……

Seyi mari……..

Tarik…………………………………………………………………………………..

Nying ja nonro……………………………………………………..

Seyi mare……………………………………….. Tarik …………………………….

Appendix V: Approval of the study

MINISTRY OF HEALTH

Telegrams: "MEDICAL", Kisumu
Telephone: 057-2020801/2020803/2020321
Fax: 057-2024337
E-mail: ercjootrh@gmail.com
When replying please quote

JARAMOGI OGINGA ODINGA TEACHING & REFERRAL HOSPITAL
P.O. BOX 849
KISUMU

Ref: ERC.1B/VOL.I/167

Date 18th March, 2015

Allan Fredrick Ogendo,
Reg. No. TM-312/2382/2013,
JKUAT.

Dear Allan,

RE: FORMAL APPROVAL TO CONDUCT RESEARCH TITLED: *"PREVALENCE AND ASSOCIATED FACTORS FOR CRYPTOSPORIDIUM INFECTION IN BOVINE AND ENVIRONMNET, ASEMBO: RARIEDA DISTRICT"*

The JOOTRH ERC (ACCREDITATION NO. 01713) has reviewed your protocol and found it ethically satisfactory. You are therefore, permitted to commence your study immediately. Note that this approval is granted for a period of one year (18th March, 2015 to 19th March, 2016). If it is necessary to proceed with this research beyond the approved period, you will be required to apply for further extension to the committee.

Also note that you will be required to notify the committee of any protocol amendment(s), serious or unexpected outcomes related to the conduct of the study or termination for any reason.

Finally, note that you will also be required to share the findings of the study in both hard and soft copies upon completion.

The JOOTRH ERC takes this opportunity to thank you for choosing the institution and wishes you the best in your endeavours.

Yours sincerely,

WILBRODA MAKUNDA,
For: SECRETARY - ERC,
JOOTRH – KISUMU.

JOOTRH ETHICS & REVIEW COMMITTEE
P. O. Box 849 - 40100
KISUMU

Appendixvi: Publication

Supplement article

PanAfrican Medical Journal

Research

Cryptosporidium infection in calves and the environment in Asembo, Western Kenya: 2015

Allan Ogendo[1,2,&], Mark Obonyo[2,3], Peter Wasswa[4], Austine Bitek[3,5], Amos Mbugua[1], Samuel Mwangi Thumbi[6]

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Guest editors: Zeinab Gura, Jane Githuku, Sara Lowther

Available online at: http://www.panafrican-med-journal.com/content/series/28/1/9/full

Abstract

Introduction: *Cryptosporidium* species, a zoonotic enteric coccidian parasite, is among the leading causes of diarrhea in children. We evaluated the prevalence of *Cryptosporidium* infections in calves, factors associated with calf infection, environmental contamination of manure by *Cryptosporidium* and factors that expose humans to zoonotic transmission in Asembo.
Methods: in a cross-sectional study conducted from January to July 2015, we collected fecal specimens from 350 randomly selected calves aged ≤ 6 months old and 187 manure samples from the same farms. We assessed farmers' knowledge about *Cryptosporidium* and collected data on characteristics using structured questionnaires. Modified Ziehl Nielsen staining was used to detect *Cryptosporidium* oocysts from calves' stool and manure. The prevalence of infected calves and 95% confidence interval (CI) were calculated. Odds ratios (OR) and 95% (CI) were calculated to identify possible factors associated with *Cryptosporidium* infection; multivariable logistic regression performed to identify factors independently associated with the presence of *Cryptosporidium*.
Results: calves' fecal *Cryptosporidium* prevalence was 8.3% (95% CI: 5.7-11.8) and 7.5% (95% CI: 4.2-12.2) in manure. Odds of infection was higher in calves with loose stool compared to those with normal stool (AOR = 6.1, 95% C.I: 2.2-16.9), calves ≤ 2 months old compared to older calves (AOR=12.7, 95% C.I: 4.5-35.8) and calves in poor sanitation compared to calves in good hygienic conditions (AOR = 9.9, 95% C.I: 3.1-30.7).
Conclusion: presence of *Cryptosporidium* species in calves and environment and reported human contact with animals increases zoonotic risk. We recommend further studies that determine specific *Cryptosporidium* species infecting animals and humans which would better estimate risk of disease transmission to humans.

Appendix VII: Computer Generated Random sampling numbers

AnimalID	HouseholdID	CompoundID	VillageID
E02045	2-100-5	2-100	2
E02046	2-107-6	2-107	2
E02047	2-109-5	2-109	2
E02048	2-127-2	2-127	2
E02049	2-140-5	2-140	2
E02050	2-143-6	2-143	2
E02051	2-147-3	2-147	2
E02052	2-149-2	2-149	2
E02053	2-153-2	2-153	2
E02054	2-154-4	2-154	2
E02055	2-155-4	2-155	2
E02056	2-183-3	2-183	2
E02057	2-202-2	2-202	2
E02058	2-209-6	2-209	2
E02059	2-41-5	2-41	2
E02060	2-58-7	2-58	2
E02061	2-96-10	2-96	2
E10001	10-124-3	10-124	10
E10002	10-159-2	10-159	10
E10003	10-171-9	10-171	10
E10004	10-179-3	10-179	10
E10005	10-18-11	10-18	10
E10006	10-188-2	10-188	10
E10007	10-198-5	10-198	10
E10008	10-198-7	10-198	10
E10009	10-2-6	10-2	10
E10011	10-20-10	10-20	10
E10012	10-25-3	10-25	10
E10013	10-324-1	10-324	10
E10014	10-331-3	10-331	10
E10015	10-331-4	10-331	10
E10016	10-333-1	10-333	10
E10017	10-39-6	10-39	10
E10018	10-53-3	10-53	10
E10019	10-80-9	10-80	10
E10020	10-87-5	10-87	10
E10021	10-94-5	10-94	10
E10022	10-96-11	10-96	10
E11154	11-283-2	11-283	11
E11155	11-103-2	11-103	11
E11156	11-105-1	11-105	11
E11157	11-125-1	11-125	11
E11158	11-157-1	11-157	11
E11159	11-163-1	11-163	11
E11160	11-202-2	11-202	11
E11161	11-21-1	11-21	11
E11162	11-78-2	11-212	11
E11163	11-215-1	11-215	11
E11164	11-256-1	11-256	11
E11165	11-260-1	11-260	11
E11166	11-287-1	11-287	11
E11167	11-303-1	11-303	11
E11168	11-259-2	11-323	11
E11169	11-146-2	11-382	11
E11170	11-153-2	11-393	11
E11171	11-190-1	11-424	11
E11172	11-229-2	11-435	11
E11173	11-133-2	11-439	11
E11174	11-183-3	11-442	11
E11175	11-495-1	11-495	11
E11176	11-524-1	11-524	11
E11177	11-55-3	11-55	11
E11178	11-162-2	11-570	11
E11179	11-62-3	11-62	11
E11180	11-8-1	11-8	11
E12181	12-143-3	12-110	12
E12182	12-121-2	12-121	12
E12183	12-125-1	12-125	12
E12184	12-155-1	12-155	12
E12185	12-178-1	12-178	12
E12186	12-179-1	12-179	12
E12187	12-181-1	12-181	12
E12188	12-110-2	12-200	12
E12190	12-24-1	12-24	12
E12191	12-161-4	12-286	12
E12192	12-17-2	12-314	12
E12193	12-101-6	12-321	12
E12194	12-34-1	12-34	12
E12195	12-35-1	12-35	12
E12196	12-58-1	12-58	12
E12197	12-61-2	12-61	12
E12198	12-64-1	12-64	12
E12199	12-90-1	12-90	12
E13023	13-10-2	13-10	13
E13024	13-110-13	13-110	13
E13025	13-127-7	13-127	13
E13026	13-142-3	13-142	13
E13027	13-144-5	13-144	13
E13028	13-18-3	13-18	13
E13029	13-188-4	13-188	13
E13030	13-2-3	13-2	13
E13031	13-203-9	13-203	13
E13032	13-208-2	13-208	13
E13033	13-221-3	13-221	13
E13034	13-222-5	13-222	13
E13035	13-235-5	13-235	13
E13036	13-236-2	13-236	13
E13037	13-246-4	13-246	13
E13039	13-268-3	13-268	13
E13040	13-3-5	13-3	13