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COLLEGE OF VETERINARY MEDICINE AND AGRICULTURE

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MASTER OF SCIENCE IN ONE HEALTH

STUDY ON *CRYPTOSPORIDIUM* IN CALVES, CHILDREN, AND DRINKING WATER SOURCES IN AKAKI KALITY, ADDIS ABABA

MSc THESIS

BY

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BISHOFTU, ETHIOPIA

Addis Ababa University
College of Veterinary Medicine and Agriculture
Department of Veterinary Microbiology, Parasitology and Poultry Health
Masters of Science in One Health

Study on *Cryptosporidium* in Calves, Children, and Drinking Water Sources in Akaki Kality, Addis Ababa

The Thesis Submitted to Addis Ababa University College of Veterinary Medicine and Agriculture, in Partial Fulfillment of the Requirement for the Degree of Master of Science in One Health

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DEDICATION

I dedicate this thesis document to my father Meribo Nebi and my mother Buja Bekeye who brought me up and nursed me with love and affection, had been the backbone of my academics, and for their unforgettable support and courage during the toughest days of my life!

STATEMENT OF THE AUTHOR

First, I declare that this thesis is my own work and is not submitted to any institution elsewhere for the grant of any academic degree, diploma or certificate and all sources of materials used for this Thesis have been properly acknowledged.

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BIOGRAPHICAL SKETCH

Abdurahman Meribo Nebi was born on April 22, 1992, in Kokosa Woreda, West Arsi Zone of the Oromia Region, Ethiopia, to his father Meribo Nebi.and his mother Buja Bekeye. He began his formal education at Kuyera Elementary School in Bokore Kebele, where he completed his primary studies in June 2005. He pursued his secondary education at Dodola Secondary School from 2006 to 2007, followed by preparatory education at Dodola Preparatory School between September 2008 and June 2009.

In 2010, he was admitted to Jimma University to study veterinary medicine and successfully graduated in 2015 with a Doctor of Veterinary Medicine (DVM) degree. Following graduation, he served as a veterinary professional at the West Arsi Zone Kokosa Woreda Livestock and Fishery Resource Development Office from April 2016 to May 2017. He then continued his public service at the Shashemene City Agricultural Office from June 2017 to October 2023.

In pursuit of advanced academic and interdisciplinary knowledge, Abdurahman joined the Master of Science in One Health program at Addis Ababa University, College of Veterinary Medicine and Agriculture, in November 2023 to present day.

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LIST OF ABBREVIATIONS

AOR	Adjusted Odds Ratio
CDC	Centers for Disease Control and Prevention
CI	Confidence Interval
DALY	Disability-Adjusted Life Year
DNA	Deoxyribonucleic Acid
EIA	Enzyme Immunoassay
ELISA	Enzyme-Linked Immunosorbent Assay
HIV	Human Immunodeficiency Virus
LM	Light Microscopy
MOH	Ministry of Health
NGO	Non-Governmental Organization
OR	Odds Ratio
PCR	Polymerase Chain Reaction
SD	Standard Deviation
SPSS	Statistical Package for the Social Sciences
WHO	World Health Organization
ZN	Ziehl–Neelsen

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ABSTRACT

Cryptosporidiosis, caused by protozoan parasites of the genus *Cryptosporidium*, poses a significant public health and veterinary challenge globally, particularly in low-resource settings with inadequate water, sanitation, and hygiene (WASH) conditions. This cross-sectional study (November 2024–June 2025) estimated the prevalence, burden, and associated risk factors of *Cryptosporidium* infection in calves, children under five, and drinking water sources (Akaki, Gefersa, and Legedadi rivers) using a One Health approach. A total of 232 calf fecal samples, 124 stool samples from children, and 60 water samples were examined using the modified Ziehl-Neelsen acid-fast staining technique. The overall prevalence was 13.4% in calves, 7.3% in children, and 60% in water samples. Significant differences in calf prevalence were observed among production systems (*p* = 0.005), body condition (*p* = 0.007), and presence of calving pens (*p* = 0.043). Calves in extensive systems had the highest infection rate (50%), and those in poor condition were most susceptible. In children, infection was significantly associated with parental illiteracy (*p* = 0.008) and animal contact (*p* = 0.049), while age and sex were non-significant. All Akaki River samples were contaminated (100%), followed by Gefersa (80%); Legedadi showed no contamination. Microscopy-based burden assessment revealed the highest oocyst loads in calves under three months and children below one year, with no high-burden cases in children. The study underscores the critical role of animal management, environmental sanitation, and public health behaviors in transmission. Integrated One Health strategies addressing human-animal-environment interactions are essential to reduce the zoonotic and waterborne burden in urban Ethiopia.

Keywords: *Akaki Kality, calves, children, Cryptosporidium*, risk factors, water sources*

1. INTRODUCTION

1.1. Background

Cryptosporidiosis is a significant public health concern caused by protozoan parasites from the *Cryptosporidium* genus. It is recognized as a major waterborne and zoonotic disease, especially in low- and middle-income countries where water, sanitation, and hygiene (WASH) infrastructure remains insufficient (Abadura *et al.,* 2022). This parasite affects a wide range of vertebrate hosts, including humans, and often leads to diarrhea, particularly in vulnerable groups such as immunocompromised individuals, young children, and newborn livestock (Palacios, 2023; Paudyal *et al.,* 2023).

Among the many species of *Cryptosporidium*, *C. parvum* and *C. hominis* are the most commonly associated with human infections. Particularly, *C. parvum* also infects calves, which highlights its zoonotic potential and underlines the importance of the human-animal-environment interface in sustaining its transmission cycle (Smith *et al.,* 2023; Gattan *et al.,* 2023). The parasite's life cycle includes the release of durable, thick-walled oocysts through feces. These oocysts are highly resistant to environmental conditions and chlorination, allowing them to persist in contaminated water, soil, and food and spread rapidly through ingestion or inhalation (Ali *et al.,* 2024; Martinez *et al.,* 2024).

Several studies have identified key risk factors that facilitate the transmission of *Cryptosporidium*. These include drinking untreated water, poor hygiene practices, open defecation, crowded living conditions, and direct or indirect contact with infected animals or contaminated environments (Jilo *et al.,* 2022; Paudyal *et al.,* 2023). In resource-limited areas, the impact of the disease is particularly severe, as communities often rely on surface water or share sources with animals, increasing the likelihood of contamination and infection (Palacios, 2023). Climate change and increased flooding events also contribute to the wider spread of oocysts in the environment (Martinez *et al.,* 2024; WHO, 2023).

In Ethiopia, while cryptosporidiosis has been detected in both humans and animals, the disease remains underreported. Studies have documented prevalence rates ranging from 2.3% to 27.8%

in calves and up to 14.8% in children. These infections are frequently associated with poor sanitation, unsafe drinking water, and zoonotic transmission (Abadura *et al.,* 2022; Manyazewal *et al.,* 2018). Despite its burden, routine diagnostic and monitoring efforts are limited. The Modified Ziehl-Neelsen (MZN) staining method is still widely used for diagnosis, although its low sensitivity means many infections likely go undetected (Gattan *et al.,* 2023).

Determinations to minimize environmental contamination have focused on livestock management strategies, such as maintaining sanitary housing, reducing overcrowding, separating young calves, and optimizing calving periods. These practices can significantly reduce the presence of oocysts in the environment (Smith *et al.,* 2023). For humans, preventive measures include promoting hand hygiene, using safe drinking water, proper food handling, and increasing public awareness especially among caregivers and children (Pumipuntu & Piratae, 2023).

Given the parasite's ability to infect multiple species and survive in the environment, adopting a One Health approach is essential. This interdisciplinary strategy connects human, animal, and environmental health to better understand and interrupt the complex transmission pathways of *Cryptosporidium* (WHO, 2023; Hatam-Nahavandi, 2019). However, in Ethiopia, there is a notable lack of One Health-based studies, particularly in urban and peri-urban settings like Akaki Kality Sub-City, where dense human populations, livestock farming, and open water sources coexist.

1.2. Statement of the Problem

Currently, there is neither a fully effective treatment nor a licensed vaccine for cryptosporidiosis in humans or animals. The parasite’s resilience, combined with a small number of oocysts are required to cause infection makes it particularly challenging to control. In Ethiopia, research on *Cryptosporidium* using a One Health framework is limited, especially in urbanizing areas where people, livestock, and shared water sources interact closely. Comprehensive knowledge on the prevalence, health impacts, and contributing risk factors across humans, animals, and water sources is urgently needed to develop integrated and effective control strategies.

1.3. Objectives of the Study

General Objective

To assess the occurrence and burden of Cryptosporidiosis in calves, children under five, and drinking water sources in Akaki Kality Sub-City, Addis Ababa, using a One Health framework.

Specific Objectives

- To estimate the prevalence of Cryptosporidiosis in calves in the study area
- To estimate the prevalence of Cryptosporidiosis in children under the age of five in the study area
- To identify major risk factors associated with the occurrence of Cryptosporidiosis in calves and children
- To estimate the burden of Cryptosporidiosis in calves, children, and water sources from a One Health perspective.

2. LITERATURE REVIEW

2.1. Etiology and Taxonomy

Cryptosporidium species belong to the family *Cryptosporidiidae*, suborder *Eimeriorina*, order *Eucoccidiorida*, subclass *Coccidiasina*, class *Sporozoasida*, and phylum *Apicomplexa* (Bhat, 2013). At present, 18 species names are linked to specific organisms, while 26 species are recognized as valid based on factors such as oocyst morphology, site of infection, genetic diversity, and host specificity across vertebrate classes (Huladeino *et al.*, 2017). Several of these species are known to infect both animals and humans, including *C. andersoni*, *C. bovis*, *C. canis*, *C. felis*, *C. hominis*, *C. meleagridis*, *C. molnari*, *C. parvum*, *C. scophthalmi*, *C. scrofarum*, *C. suis*, and *C. xiaoi*. In calves, the most frequently identified species are *C. parvum*, *C. bovis*, *C. andersoni*, and *C. ryanae*; however, only *C. parvum* is known to cause clinical disease in neonatal calves, while older animals typically shed oocysts without exhibiting symptoms (Thomson, 2017). The list of confirmed spp. based on biological, molecular and morphological is shown in (Table 1).

Table 1: Valid species of C*ryptosporidium*

Species of	Major hosts	Zoonotic status
C. andersoni	Calves	Yes
C. suis	Pigs	Yes
C. bovis	Calves	Yes
C. baileyi	Birds	No
C. canis	Dogs	Yes
C. cuniculus	Rabbits	Yes
C. felis	Cats	Yes
C. galli	Birds	No
C. hominis	Humans	Most common in human
C. meleagridis	Human and birds	Yes
C. molnari	Fish	No
C. muris	Rodents	Yes
C. parvum	Ruminants	Yes
C. ryanae	Calves	No
C. scrofarum	Pigs	Yes
C. ubiquitum	Primates, ruminants, and rodents	Yes

Source:(Huladeino *et al.,* 2017)

2.2. Infectious life cycle and pathogenesis of

Cryptosporidium follows a direct, monoxenous life cycle, meaning it completes all stages of development within one host (Ali *et al.,* 2024). The primary objective of this cycle is to generate oocysts durable, resistant forms capable of withstanding extreme environmental conditions. These oocysts are shed in the feces of infected individuals and can contaminate environments like soil, pastures, and water bodies, facilitating transmission through the fecal-oral route (Zahedi *et al.,* 2020).

When another host ingests contaminated food or water, excystation begins in the gastrointestinal tract. This process is triggered by stomach acid, bile salts, and digestive enzymes like trypsin, leading to the release of four infectious sporozoites from each oocyst (Martinez *et al.,* 2024). Sporozoites rapidly adhere to and penetrate the intestinal epithelial cells by utilizing specialized apical organelles, rhoptries, micronemes, and dense granules. These organelles are essential for breaching the host cell and forming a parasitophorous vacuole, which encloses the parasite. This vacuole not only protects the parasite from the host's immune defenses but also enables it to absorb nutrients directly from the host cell's cytoplasm (Chalmers *et al.,* 2023).

Following invasion, the parasite undergoes asexual multiplication (merogony). Initially, sporozoites develop into trophozoites, which then mature into Type I meronts. These meronts produce multiple merozoites, which either reinvade nearby epithelial cells to continue asexual replication or transition into Type II meronts, signaling the start of the sexual phase (gametogony) (Widmer *et al.,* 2023).

During gametogony, Type II meronts release merozoites that differentiate into macrogamonts (female gametes) and microgamonts (male gametes). Fertilization between these gametes results in the formation of a zygote, which then undergoes sporogony to form new oocysts. Two types of oocysts are produced: First, Thin-walled oocysts, which remain inside the host and can cause autoinfection, estimated to constitute around 20% of the total. Second, Thick-walled oocysts, which are excreted in large numbers through feces and are responsible for environmental transmission and long-term survival (Gattan *et al.,* 2023; Smith *et al.,* 2023).

The widespread presence of both humans and animals in shared ecosystems facilitates the transmission of *Cryptosporidium,* enhancing its outbreak potential (Jones *et al.,* 2023); especially, the entire life cycle from ingestion to the release of new oocysts can be completed in as little as 12 to 14 hours, contributing to the parasite’s rapid spread and persistence (Figure 1).

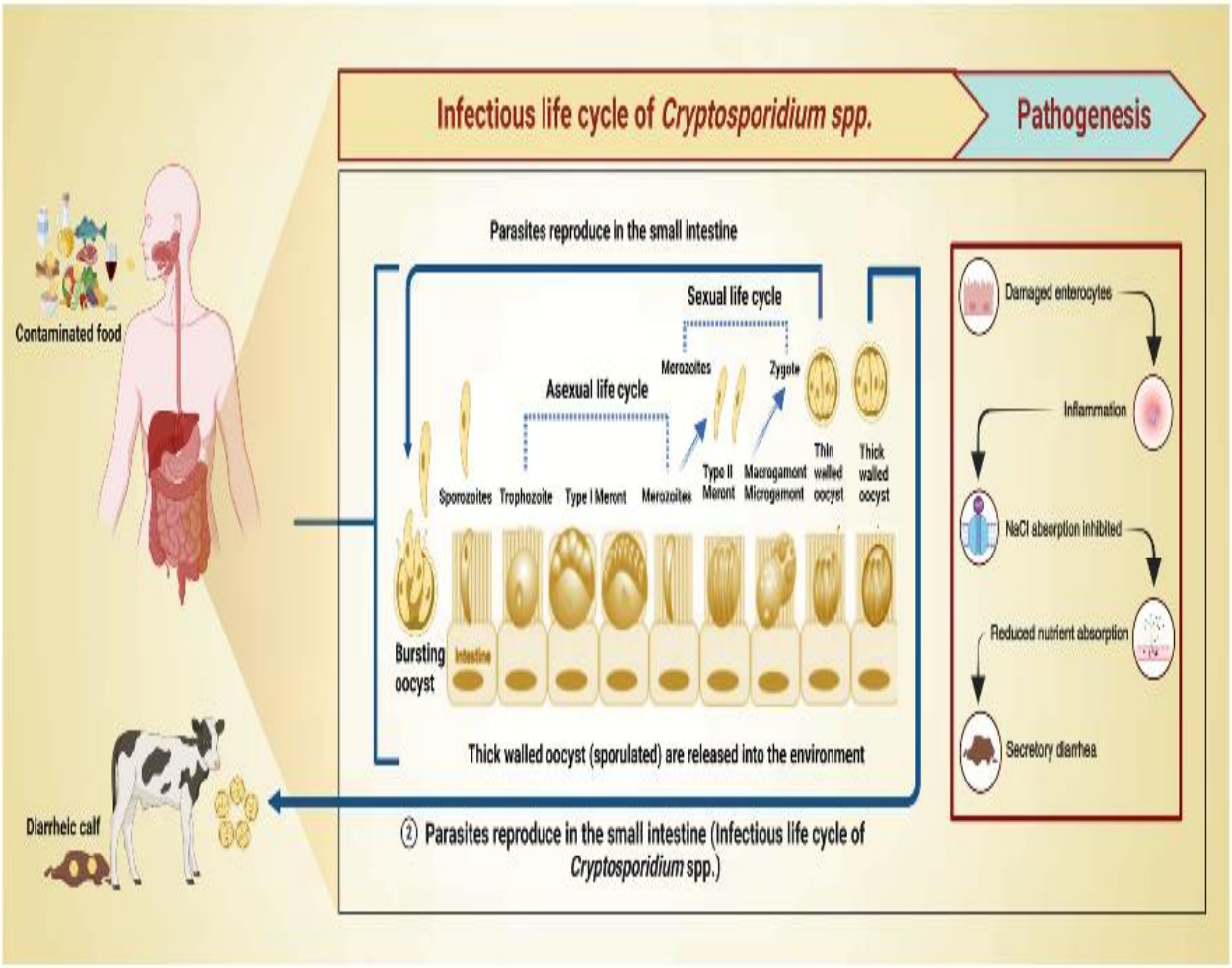

Figure 1: Infectious life cycle and pathogenesis of *Cryptosporidium* species**.**

Source: (Ali *et al.*, 2024).

2.2.2. Replication Dynamics and Pathogenesis of *Cryptosporidium* Infection

This rapid replication cycle allows successive generations to develop in a short time, contributing significantly to their persistence and spread (Martinez *et al.*, 2024; Chalmers *et al.*, 2023). In cattle, the seriousness of infection can vary widely, with oocyst counts ranging from as few as 2 to over 1,000 per gram of feces. Oocyst shedding typically continues for 3 to 15 days after the initial infection (Gattan *et al.*, 2023). This mass excretion is one of the most critical drivers of environmental contamination, especially in regions with high livestock densities.

Humans also contribute significantly to environmental oocyst load. Infected individuals can excrete between 10^5 to 10^7 oocysts per gram of feces, further contaminating water sources and increasing the risk of waterborne outbreaks (Smith *et al.,* 2023; Zahedi *et al.,* 2020).

Cryptosporidiosis usually has an incubation period of about 3 to 15 days, with most people beginning to show symptoms roughly 5 to 7 days after being exposed to the parasite. These symptoms can persist for several weeks (Ali *et al.,* 2024). The pathological effects are primarily localized to the intestinal epithelium, where sporozoites invade the brush border of epithelial cells. Histological examination often reveals epithelial cell death (necrosis), shortening or blunting of villi, hyperplasia of intestinal crypts, and disruption of microvilli. Infected cells may exhibit elongated or fused microvilli, likely resulting from cytoskeletal changes triggered by the parasite (Gattan *et al.,* 2023; Chalmers *et al.,* 2023).

These changes impair normal nutrient absorption and stimulate an immune response. Infected individuals experience an inflammatory reaction involving cytokines and prostaglandins, which increase chloride secretion while reducing sodium absorption in the gut. This imbalance results in secretory diarrhea, a manifestation of the disease (Korpe *et al.,* 2023). Those with compromised immune systems, such as malnourished children or individuals with HIV/AIDS, are especially vulnerable and tend to experience more severe and prolonged illness.

While the gastrointestinal tract is the primary target of infection, extra-intestinal manifestations have been reported, particularly in immunocompromised patients. In such cases, *Cryptosporidium* may invade the biliary tract, liver, pancreas, and even the respiratory system and sinus tissues (Sponseller *et al.*, 2021; Carter *et al.,* 2020).

Clinically, *Cryptosporidium* infection is typically marked by profuse watery diarrhea, abdominal cramping, nausea, fatigue, and often significant weight loss. In healthy individuals, symptoms usually resolve on their own within one to two weeks. However, in immunosuppressed individuals such as those living with HIV/AIDS or undergoing immunosuppressive therapy ofthe

infection can become chronic, spread beyond the intestines, and lead to life-threatening complications (Chalmers *et al.,* 2023; Abadura *et al.,* 2022).

2.3. Epidemiology

2.3.1. Global Prevalence and Geographic Distribution of Cryptosporidiosis

Worldwide, the rate of Cryptosporidium infection in pre-weaned calves varies greatly, with reported cases ranging from as low as 3.4% to as high as 96.6%, depending on factors such as location, management practices, and diagnostic methods used (Table 2).

Table 2: Prevalence of Cryptosporidiosis in different parts of the world

Region/Country	**Prevalence Rate (%)**	**Reference/Study Year**
Ethiopia	7.7 - 31.4	(Mekonnen , 2020)
Nigeria	10 – 35	(Ogunjimi , 2019)
Kenya	14.5	(Ntonifor , 2021)
South Africa	9.1 - 15.9	(Hendricks , 2018)
Uganda	15.5	(Nakalembe , 2022)
India	6.4 - 20.4	(Nath , 2021)
Thailand	3.5	(Khan , 2022)
USA	0.5 - 5.5	(Fayer , 2019)
UK	0.1 - 2.4	(Chalmers , 2020)
Brazil	5.2	(Silva , 2018)

2.3.2. Modes of Transmission and Sources of Infection

Cryptosporidiosis spreads mainly in two ways: through zoonotic transmission, where the infection passes from animals to humans, and through anthroponotic transmission, where it spreads from one person to another. The infection typically occurs when a person ingests or comes into contact with infectious oocysts, which are highly resilient and capable of surviving

for extended periods in diverse environments such as soil, food, and especially water (Zahedi *et al.*, 2021). These hardy oocysts, each containing four infective sporozoites, are the primary agents of transmission once they enter a susceptible host (Smith *et al.*, 2023).

The main way cryptosporidiosis spreads through the fecal-oral route. This happens when oocysts released in the feces of infected people or animals are accidentally swallowed by others, often through contaminated food, water, or hands. This route is particularly relevant in settings with close human contact and limited hygiene, including daycare centers, hospitals, and recreational water environments like pools and waterparks (Gharpure *et al.*, 2020).

Indirect transmission also plays a major role, occurring through contaminated food, drinking water, and inanimate objects (fomites) such as clothing, footwear, or farming tools that come into contact with infected fecal matter. One of the greatest public health challenges lies in waterborne transmission, as *Cryptosporidium* oocysts are resistant to conventional chlorination techniques used in water treatment, making both treated and untreated water sources potential vehicles for infection (Fayer & Xiao, 2020). In rare but concerning cases, aerosolized oocysts can be inhaled, leading to respiratory infections especially in immunocompromised individuals and young children (Sponseller *et al.*, 2021).

Environmental and seasonal factors also influence transmission patterns. Heavy rainfall and flooding events can wash oocysts from animal pastures or sewage-contaminated areas into rivers, lakes, and municipal water systems, elevating the risk of outbreaks (Martinez *et al.*, 2024). Contaminated food items have also been linked to outbreaks, particularly those involving unpasteurized milk. Among foodborne outbreaks, unpasteurized milk (40.9%) was the most commonly implicated sources (Gharpure *et al.*, 2019; Khan *et al.*, 2020).

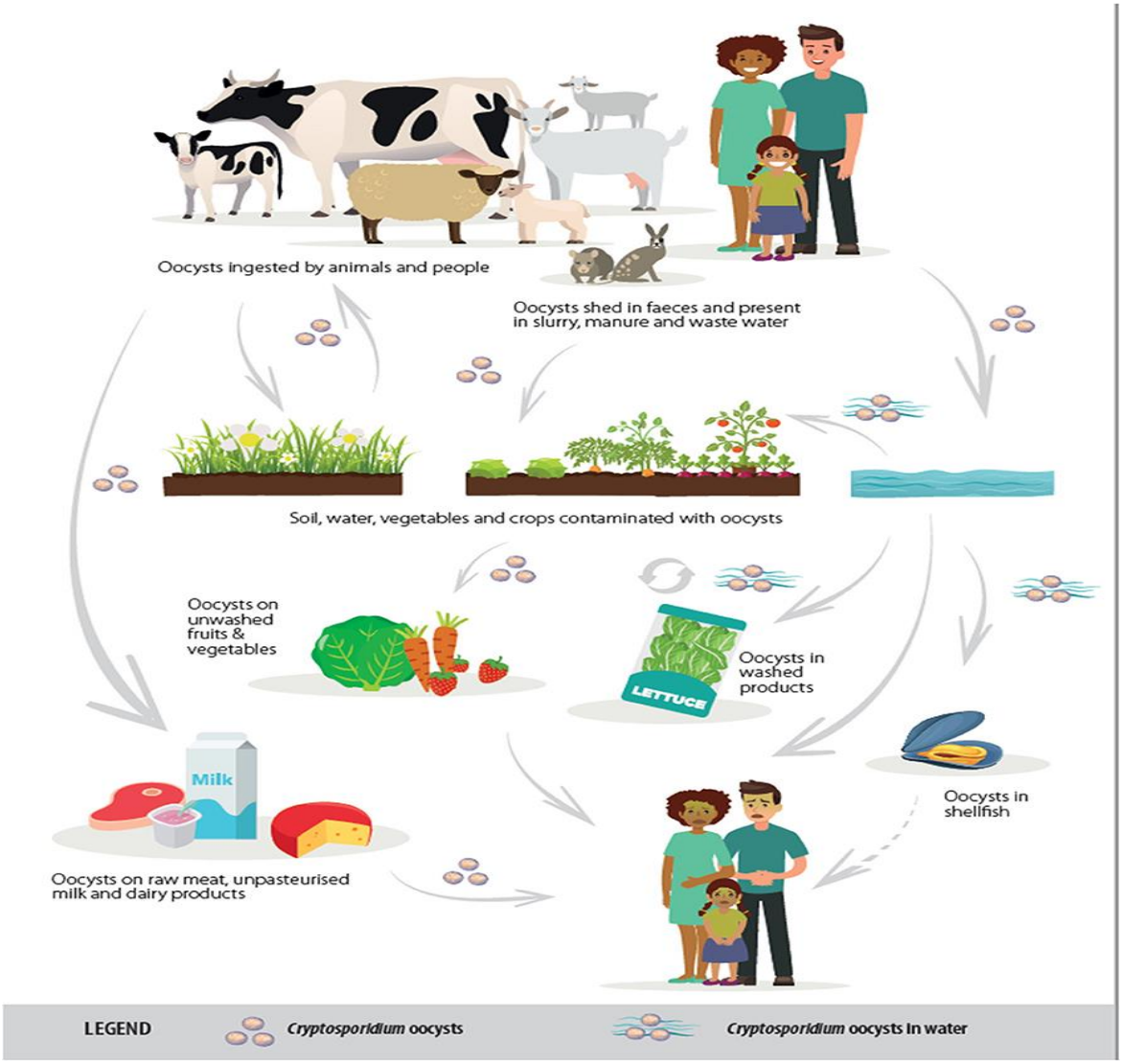

Figure 2: Transmission routes of *Cryptosporidium* spp.

Source: (Robertson, 2020).

2.4. **Clinical Signs and Potential Risk Factors of Cryptosporidiosis**

Cryptosporidiosis is a disease influenced by a combination of host, environmental, and socioeconomic factors. As outlined by the CDC (2024), individuals with weakened immune systems, such as those living with HIV/AIDS or undergoing immunosuppressive treatment, are particularly vulnerable. Other contributing factors include close contact with infected animals, younger age, male gender, and living in conditions of poverty or overcrowding. Environmental

factors such as poor water quality, seasonal variation especially during the rainy season and natural disasters like floods also increase the risk of transmission. Clinically, the infection often presents with a range of gastrointestinal symptoms, including watery diarrhea, abdominal pain, nausea, and vomiting. In more severe or prolonged cases, especially among vulnerable groups, it may lead to fever, dehydration, fatigue, and significant weight loss (Figure 3). Recognizing these overlapping risk factors and symptoms is essential for early detection and effective intervention, particularly in resource-limited settings where the burden of disease is often greatest (CDC, 2024).

Age is a major risk factor in the development and progression of the disease. Populations at the extremes of age namely infants, pre-weaned calves, and elderly individuals over 75 years are more susceptible due to underdeveloped or weakened immune systems (Chalmers *et al.,* 2023). Additionally, people with compromised immunity, including those with HIV/AIDS, cancer patients on chemotherapy, and organ transplant recipients, are at heightened risk. Particularly, individuals with CD4+ T-cell counts below 50 cells/mm³ are more likely to develop severe, persistent, and even disseminated forms of Cryptosporidiosis (Sponseller *et al.,* 2021).

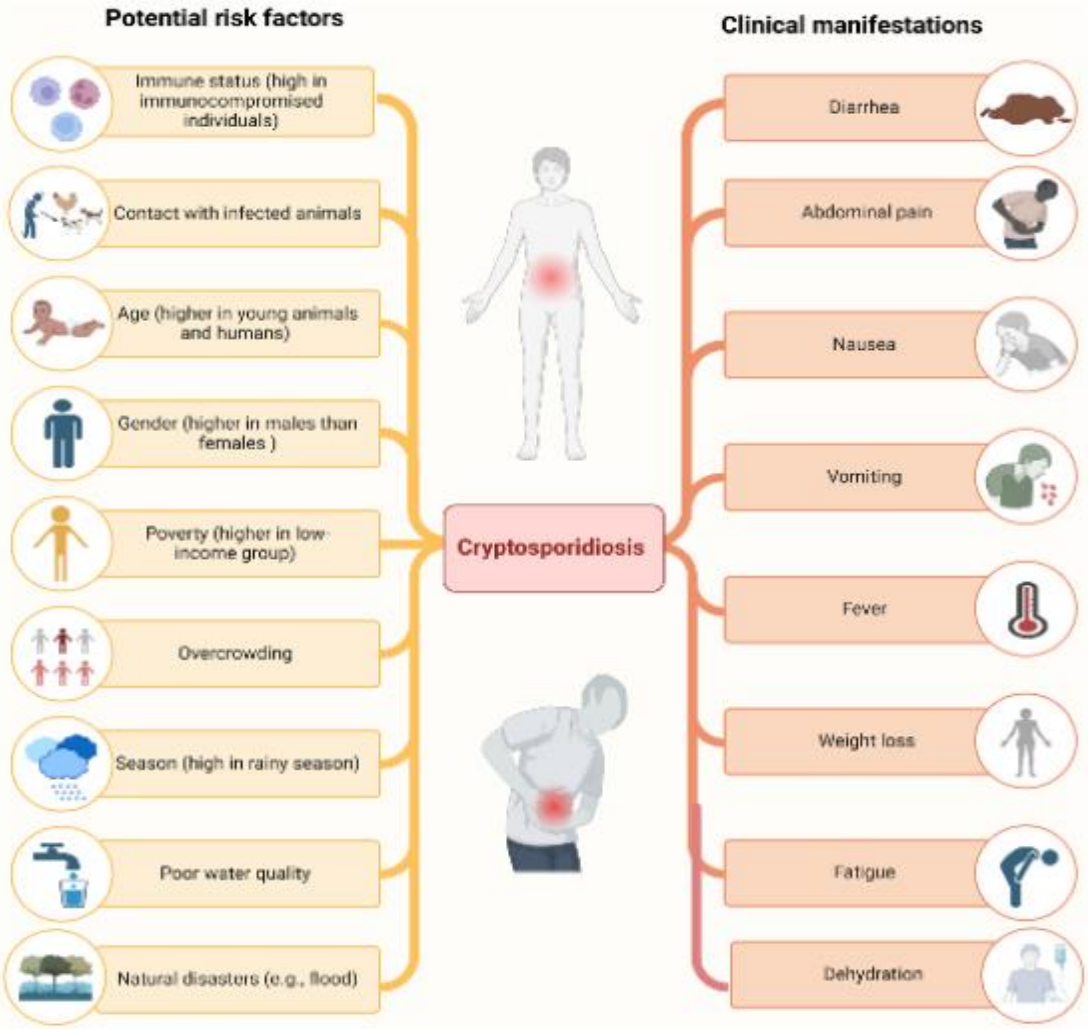

Figure 3: Risk factors and clinical manifestations associated with Cryptosporidiosis.
Source: (CDC, 2024).

2.4.1. Clinical Signs in Calves

Cryptosporidiosis is a leading cause of neonatal enteritis in calves, with clinical cases most frequently occurring during the first three weeks of life. While adult cattle may experience subclinical infections, the most severe and clinically apparent forms of the disease are typically seen in calves aged between 1 and 3 weeks. This heightened susceptibility is largely due to their underdeveloped immune systems (Gattan *et al.*, 2023).

Affected calves often present with profuse, watery diarrhea, which may be accompanied by signs such as lethargy, reduced appetite, dehydration, and, in severe cases, death particularly when complicated by secondary bacterial infections or insufficient fluid therapy (Zhang *et al.,* 2023). Diarrhea typically lasts between 5 and 14 days, though the shedding of oocysts in feces may continue even after clinical signs subside. Importantly, the presence of oocysts is not always directly linked to the severity of diarrhea, as asymptomatic shedding is common, especially in older or sub clinically infected animals (Chalmers *et al.,* 2023).

Beyond acute gastrointestinal symptoms, certain Cryptosporidium species, notably *C. andersoni*, have been implicated in long-term health and productivity issues in adult cattle. Chronic or repeated infections can result in decreased weight gain, reduced feed conversion efficiency, and lower milk yields. These impacts underscore the economic significance of early-life infections and highlight the importance of effective prevention and management strategies in livestock production systems (García-Livia *et al.,* 2021).

2.4.2. Clinical Signs in Humans

In humans, Cryptosporidium is a recognized cause of acute gastrointestinal illness individuals with weakened immune systems, and populations living in areas with inadequate water sanitation. The infection most commonly presents with profuse, watery diarrhea, accompanied by abdominal cramps, nausea, low-grade fever, vomiting, and general discomfort (Palacios *et al.*, 2023). In children, especially in low-resource settings, prolonged diarrheal episodes can lead to severe dehydration, malnutrition, growth delays, and even death when oral rehydration therapy is unavailable or insufficient (Chalmers *et al.*, 2023).

Emerging research has also revealed the potential for respiratory Cryptosporidiosis, with symptoms such as persistent cough, difficulty breathing, and sinusitis being reported in severely immunocompromised patients. These symptoms are believed to result from either inhalation of oocysts or systemic spread through the bloodstream. This expanding clinical profile emphasizes

that Cryptosporidiosis is not solely a gastrointestinal illness, but a multifaceted disease with significant implications for vulnerable populations (Ryan *et al.,* 2021).

2.5. Diagnosis of Cryptosporidiosis

Cryptosporidiosis essential for effective patient management, outbreak control, and public health surveillance. Given the disease's nonspecific clinical presentation most notably, profuse watery diarrhea laboratory confirmation is essential to distinguish it from other common causes of acute gastroenteritis, including *Giardia lamblia*, *Entamoeba histolytica*, *Escherichia coli*, rotaviruses, and *Salmonella* species (Chalmers *et al.,* 2023).

Currently, a range of diagnostic approaches is used to confirm infection, including microscopic examination, antigen detection assays, immunological methods, and molecular techniques. These tools vary in their sensitivity, specificity, cost, and practicality depending on the clinical setting, with each offering unique advantages and limitations in the detection of *Cryptosporidium* spp.

2.5.1. Microscopic Method of Examination

Microscopy remains a valuable and cost-effective method for detecting *Cryptosporidium*, especially in resource-limited regions. The modified Ziehl-Neelsen (mZN) acid-fast staining technique is the most widely used and is often regarded as the gold standard for identifying oocysts in fecal samples. Under oil immersion (×100 magnification), stained oocysts typically appear as pink to red, round to oval structures measuring approximately 4–6 µm in diameter, set against a blue background. The staining procedure involves fixing thin fecal smears with methanol, staining with carbol-fuchsin, decolorizing with acid-alcohol, and applying a counterstain, such as methylene blue or malachite green, for contrast (Widmer *et al.,* 2023).

Cryptosporidium oocysts appear as bright red or pink against a blue background, due to their acid-fast properties (Figure 4). This contrast makes them easier to spot under the microscope. Although mZN is practical and accessible, it has only moderate sensitivity and cannot distinguish

between species or genotypes. Nonetheless, it remains an important tool for routine diagnosis where advanced molecular techniques are not available (Davies and Chalmers, 2009).

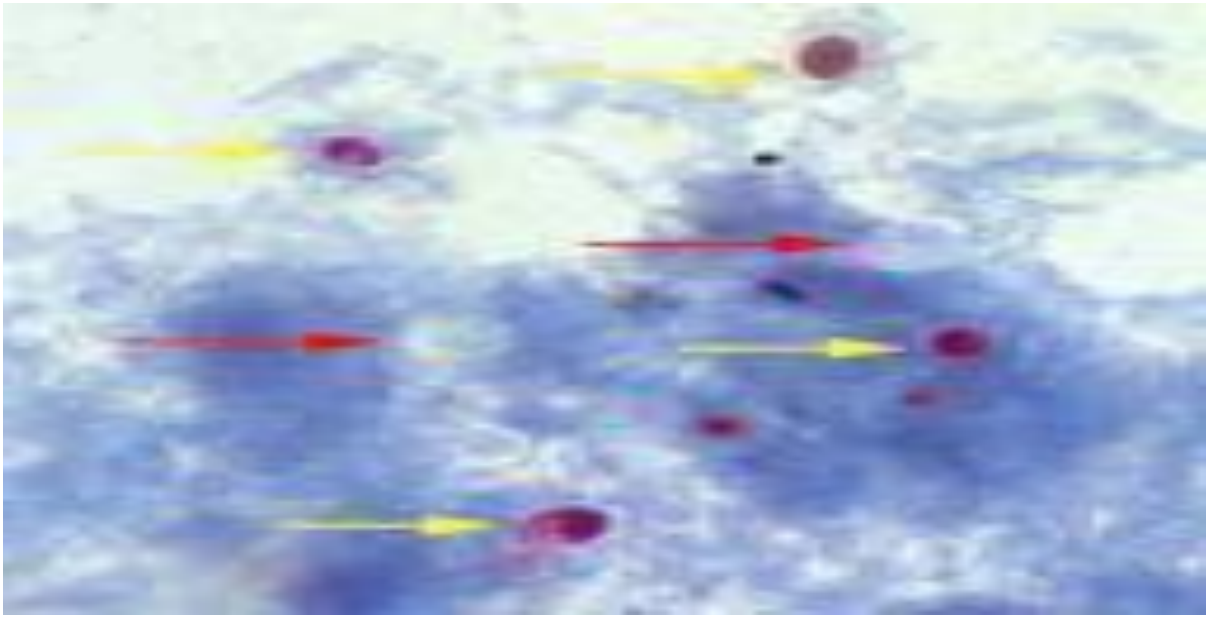

Figure 4: Oocysts of *Cryptosporidium* spp. stained by: the modified Ziehl-Neelsen stain**.**
Sources: (Davies and Chalmers, 2009).

2.5.2. Antigen Detection Methods

The use of immunoassay-based diagnostic methods has significantly enhanced the speed and sensitivity of detecting Cryptosporidium. Two commonly utilized tools, lateral flow immunochromatographic tests (ICTs) and enzyme-linked immunosorbent assays (ELISAs), allow for quick identification of *C. parvum* antigens in fecal samples. Recent studies show that these methods outperform traditional microscopy in terms of sensitivity and ease of use, making them particularly beneficial for field diagnostics and large screening programs. These antigen detection kits are especially valuable in veterinary settings for diagnosing calf diarrhea and have been found to be highly consistent with PCR-based diagnostic techniques (Amoah *et al.*, 2021).

2.5.3. Immunological and Molecular Methods

Immunological methods, such as direct fluorescent antibody (DFA) tests and ELISAs, have become increasingly popular in clinical diagnostics for *Cryptosporidium*. DFA uses monoclonal antibodies labeled with fluorescent dyes, offering greater sensitivity and specificity than traditional staining methods. However, this technique requires access to a fluorescence microscope and skilled personnel, which can limit its accessibility (Ryan *et al.*, 2021). While effective, DFA and similar tests cannot often differentiate between *Cryptosporidium* species a limitation in zoonotic disease surveillance where species identification is crucial.

PCR-based diagnostics offer numerous benefits, including high throughput, consistent results, and suitability for both clinical and environmental sample analysis. As such, they are now regarded as the gold standard, especially in molecular epidemiology and outbreak investigations (Zahedi *et al.*, 2021).

2.6. Treatment

Ongoing research has shown that effective drug treatments for Cryptosporidiosis remain limited, particularly for individuals with weakened immune systems and young animals. Medications such as azithromycin, paromomycin, and rifaximin have undergone testing; however, these therapies typically offer only limited alleviation. While they assist in decreasing oocyst shedding, they do not completely eradicate the infection (Zahedi *et al.*, 2021; Chalmers *et al.*, 2023).

2.6.1. Treatment in Calves

Halofuginone lactate is the only approved medication in the EU for preventing Cryptosporidiosis in newborn calves. For the best results, it needs to be given within the first 24 to 48 hours after birth and is not effective if the calf has shown symptoms for more than a day (Gattan *et al.*, 2023).

Additionally, paromomycin, an aminoglycoside antibiotic, has shown potential in reducing oocyst shedding and diarrhea in infected calves when administered orally at 100 mg/kg twice daily over 11 days (Lacasta *et al.,* 2021). Decoquinate and other coccidiostats have been tested but with limited efficacy. More promising, recent trials using bumped kinase inhibitors (BKIs) have demonstrated reductions in oocyst shedding in experimentally infected calves, representing a potential future therapy (Love *et al.,* 2022).

2.6.2. Treatment in Humans

Nitazoxanide is currently the sole FDA-approved medication for treating Cryptosporidiosis in humans. It is most beneficial for individuals with a healthy immune system, as it can help shorten symptom duration and decrease oocyst shedding. However, its effectiveness is notably reduced in immunocompromised patients, such as those with HIV/AIDS or receiving immunosuppressive treatment (Zahedi *et al.,* 2021).

Supportive care is essential for vulnerable populations, particularly those at high risk. Initially, oral rehydration solutions are used to address dehydration, but in more severe situations, intravenous fluids may be necessary. Nutritional support is equally important, especially for children and malnourished individuals. In cases of intestinal damage, incorporating supplements that contain medium-chain triglycerides can enhance nutrient absorption and overall recovery (Palacios *et al.,* 2023).

2.7. Prevention and Control Measures of Cryptosporidiosis

2.7.1. Prevention and Control in Calves

Maintaining good farm management practices is key to controlling the spread of . This includes routinely cleaning out manure and dirty bedding, keeping calving areas sanitized, and using disinfectants like hydrogen peroxide or ammonia-based solutions to reduce contamination (Chalmers *et al.,* 2023). Reducing animal density, segregating neonates from older animals, and

shortening the calving season can also minimize transmission. No commercial vaccines currently exist for *Cryptosporidium*; however, passive immunity via colostrum is under investigation. Immunization of pregnant cows with recombinant antigens has shown promise in enhancing antibody levels in colostrum, which may reduce oocyst shedding and clinical severity in calves (González *et al.,* 2021).

2.7.2. Prevention and Control in Humans

Preventing Cryptosporidiosis in people hinges on maintaining good hygiene and sanitation practices. Regular handwashing, steering clear of unfiltered water, and safely handling raw food can significantly reduce the risk of infection. Additionally, anyone experiencing diarrhoea should refrain from using public pools to help prevent the spread of the parasite through shared water (Ryan *et al.,* 2021).

Certain chemical methods like ozone, UV light, chlorine dioxide, and hydrogen peroxide vapour can help destroy *Cryptosporidium* oocysts on surfaces and in water. However, regular chlorination doesn't work well against them. For home use, boiling water is still one of the most dependable ways to ensure it's safe to drink (Cacciò & Ryan, 2021).

2.8. Cryptosporidium of drinking water sources

Research highlights the increased risk of *Cryptosporidium* contamination when livestock are kept near water sources, stressing the importance of managing both water and animal populations together (Paudyal *et al.,* 2023). This parasite is particularly concerning for drinking water safety because it is resistant to standard disinfection methods and can survive under various environmental conditions (Smith *et al.,* 2023). Furthermore, climate change and extreme weather events could exacerbate contamination risks by overwhelming water treatment systems and leading to more runoff into water supplies (Martinez *et al.,* 2024)

Cryptosporidium oocysts found in contaminated drinking water (Figure 5), emphasizing the parasite's ability to spread through waterborne transmission.

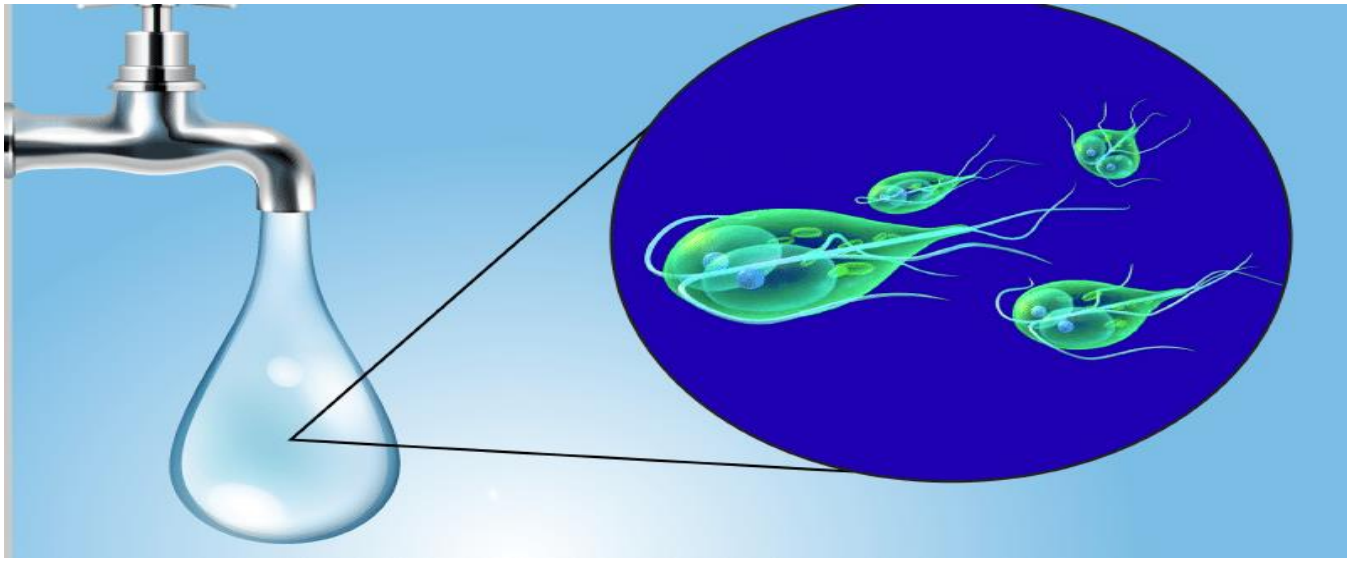

Figure 5: *Cryptosporidium* Parasites in drinking water
Sources: *https://aurigaresearch.com/water-testing*.

2.8. Burden of *Cryptosporidium*

Cryptosporidiosis is a significant parasitic disease that is transmitted from animals to humans and through contaminated water sources. It poses a major threat to public health, livestock production, and overall socioeconomic development. This disease is especially prevalent in low- and middle-income countries, where access to clean water, proper sanitation, and good hygiene practices are limited. Cryptosporidium is a leading cause of severe diarrhoea in children under five worldwide, second only to rotavirus. In areas where the disease is endemic, it is responsible for a large proportion of diarrheal-related deaths in young children (Checkley *et al.,* 2021).

2.8.1 At-Risk Populations and Transmission Risks

Populations at increased risk include immunocompromised individuals (e.g., people with HIV/AIDS), young children, the elderly, and individuals exposed to contaminated water or

infected animals. Occupational and recreational exposure, such as swimming in inadequately chlorinated pools, handling infected calves, or working in veterinary and childcare settings, significantly contributes to human infection (Palacios *et al.,* 2023; Smith *et al.,* 2023). In immunocompromised individuals, chronic and severe diarrhea can result in dehydration, malnutrition, and death, while infections in immunocompetent individuals tend to be self-limiting (Chalmers *et al.,* 2023).

2.8.2 Diagnostic and Quantitative Burden

Microscopic examination using modified Ziehl-Neelsen staining remains a basis for diagnosis in both human and veterinary medicine. Oocyst quantification under high-power fields (HPF) serves as a representation for infection intensity, with burden levels classified as low (<5 oocysts/HPF), moderate (5–10 oocysts/HPF), or high (>10 oocysts/HPF), aiding in clinical and epidemiological evaluations (Gattan *et al.,* 2023).

2.8.3 Burden in Calves and Livestock

C. parvum is the predominant species infecting neonatal calves, with a global prevalence estimated at 21.9% (Zhang *et al.,* 2023). Calves infected with a particular parasite can release millions of oocysts into their surroundings each day, contaminating their environment and posing a risk of transmission to humans, often linked to poor hygiene, feeding practices, and animal contact (Gattan *et al.,* 2023; Paudyal *et al.,* 2023).

In high infection rates in calves were observed during the second week of life, emphasizing the need for early intervention on dairy farms (Nováková *et al.,* 2023). Economic modeling in dairy operations has estimated many losses of calf, primarily due to treatment costs, growth retardation, and increased mortality (Widmer *et al.,* 2023).

2.8.4 Burden in Humans and Waterborne Risk

In humans, Cryptosporidiosis continues to be a major contributor to childhood morbidity. A study in Ecuador found a prevalence of 14.3% among symptomatic children, with significant environmental contamination as water samples revealed oocyst counts of up to 5 per 100 mL (Palacios *et al.*, 2023). Globally, *Cryptosporidium* is implicated in more than 50% of waterborne parasitic outbreaks. These outbreaks are often traced to failures in water treatment and fecal contamination from livestock and wildlife (Smith *et al.*, 2023).

Children especially those under the age of four and older adults are particularly vulnerable to severe illness. In many cases, children become infected around the time of weaning or shortly afterward, when their exposure to contaminated food or water may increase (Chalmers *et al.*, 2023). Early-life infection is also associated on growth stunting and impaired cognitive development (Checkley *et al.,* 2021).

2.9. Status of Cryptosporidiosis in Ethiopia

Cryptosporidiosis remains a significant yet under-researched parasitic infection in Ethiopia, affecting both livestock and humans. Due to poor sanitation infrastructure, low levels of awareness, and limited diagnostic capacity, the burden of this protozoan disease is likely underestimated, especially in rural communities where contact between humans and animals is common (Wegayehu *et al.*, 2023).

2.9.1. Status of Cryptosporidiosis in Calves in Ethiopia

Ethiopia has a cattle population exceeding 50 million, with many animals reared by smallholder farmers across a range of agro-ecological zones. Despite this large population, research on the spread and impact of *Cryptosporidium* in calves remains limited. Existing studies, mainly cross-sectional, have shown that infection rates vary by region. For instance, one study conducted on 40 dairy farms in central Ethiopia reported a prevalence rate of 17.6% (Abebe *et al.*, 2008), while

a study conducted in Haramaya, eastern Ethiopia, reported a prevalence of 27.8% (Regassa *et al.*, 2013). Similarly, Ayele *et al.* (2018) identified an 18.6% prevalence among 360 calves sampled in north western Ethiopia.

Molecular analyses have confirmed the presence of multiple *Cryptosporidium* species in Ethiopian calves, including *C. parvum*, *C. andersoni*, *C. bovis*, and *C. ryanae* (Wegayehu *et al.*, 2016; Mengistu *et al.*, 2022). These species are not only pathogenic but also carry zoonotic potential, particularly *C. parvum*, which is commonly shared between calves and humans. Collectively, the reported prevalence of bovine Cryptosporidiosis in Ethiopia ranges from 2.3% to 27.8%, depending on region, age group, and diagnostic method (Abebe *et al.*, 2008; Ayele *et al.*, 2018; Mengistu *et al.*, 2022).

2.9.2. Status of Human Cryptosporidiosis in Ethiopia

Human Cryptosporidiosis in Ethiopia remains a neglected public health issue despite its notable burden in children and immunocompromised individuals. Risk factors commonly reported include poor hygiene practices, consumption of untreated water, and close contact with livestock, particularly young calves. Recent studies have highlighted a prevalence range of 7.3% to 12.2% in apparently healthy children, while in symptomatic or hospitalized children, prevalence ranges from 5.6% to 14.8% (Teshome *et al.*, 2014; Wegayehu *et al.*, 2013; Manyazewal, 2017) (Table 3).

Table 3: Prevalence of Cryptosporidiosis in Humans in Ethiopia

Study Area	Study Group	Prevalence	Reference
Jimma Hospital	Under-5 children	3.3%	Gebru & Girma (2000)
Lege Dini	Healthy children	12.2%	Ayalew *et al*. (2008)
Nine regions	Various ages	7.6%	Adamu (2010)
Pawi District	Healthy children	8.1%	Tigabu (2010)
North Shewa Zone	Healthy children	7.3%	Wegayehu *et al.* (2013)
Yirgalem Hospital	Patient children	14.8%	Teshome *et al*. (2014)
Addis Ababa & Environs	HIV+ individuals, public	9%	Manyazewal (2017)

2.10. One Health Approach to Combating Cryptosporidiosis

The One Health framework acknowledges that human, animal, and environmental health are inextricably linked. Its application is critical in controlling and preventing Cryptosporidiosis, as the disease affects multiple species and spreads across ecological boundaries (Chalmers *et al.*, 2023). Outbreaks of zoonotic cryptosporidiosis have been linked to close contact with farm animals, drinking raw milk contaminated with the parasite, and using water that hasn't been properly treated (figure 6).

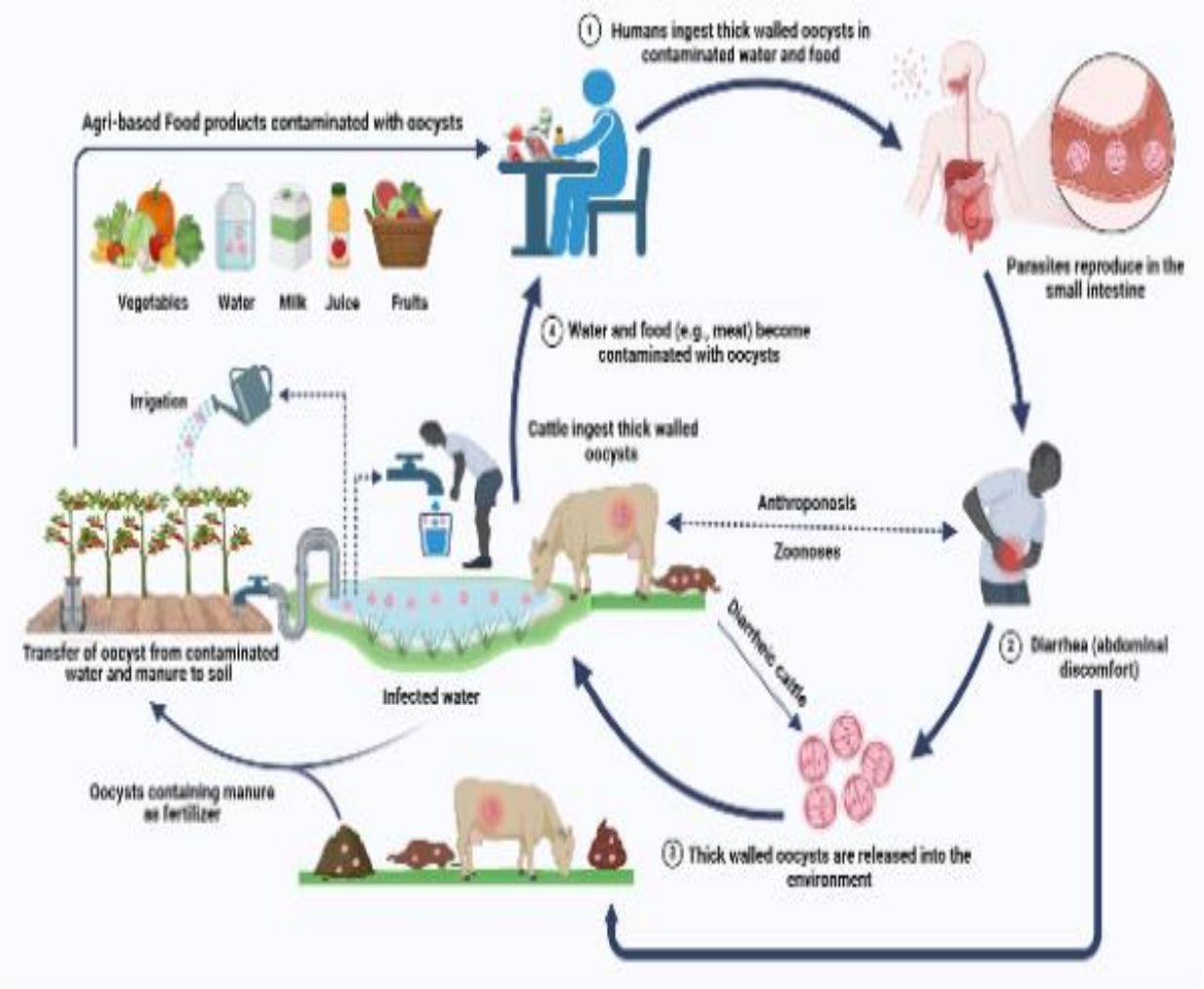

Figure 6: A One Health perspective on transmission *Cryptosporidium*.

Source: (Hatam-Nahavandi, 2019).

3. MATERIALS AND METHODS

3.1. Description of study area

The study was conducted from November 2024 to June 2025 across five districts (Woredas) in Akaki Kality Sub-City, located in the southern part of Addis Ababa, Ethiopia (Addis Ababa City Administration, 2018). This sub-city is mostly flat and situated in a lowland area, with an average elevation of approximately 2,200 meters above sea level (Ethiopian Mapping Authority, 2017). With an estimated population of 255,348, Akaki Kality is considered moderately populated for its geographical size (Central Statistical Agency, 2022). The sub-city continues to experience population growth, largely driven by migration from rural regions and smaller towns, as individuals move in search of employment opportunities in the area's expanding industrial sector (World Bank, 2021). Population density varies significantly, with urban centers being more overcrowded (Addis Ababa Bureau of Urban Development, 2019).

Despite its urbanizing trend, Akaki Kality maintains a substantial livestock population, reflecting its mixed urban peri-urban location. According to the most recent agricultural data, the sub-city livestock population approximately 7,426 cattle, 3,399 sheep and 716 goats, 151 horses, 522 donkeys and 408,368 chickens (Akaki Kality Farmers and City Agricultural Development Office, 2025).

Sanitation conditions across Akaki Kality vary by neighborhood. While residents in planned urban areas often benefit from sewage infrastructure, those in informal or peri-urban settlements typically depend on pit latrines (Ministry of Health, Ethiopia, 2021). Environmental sanitation is further challenged by industrial waste, particularly from tanneries and manufacturing facilities situated near the Akaki River, which contribute significantly to local pollution concerns (Environmental Pollution Journal, 2020). In response, public health agencies have initiated programs aimed at expanding sanitation coverage in underserved communities (WHO, 2021).

The primary source of drinking water in the sub-city is piped municipal water managed by the Addis Ababa Water and Sewerage Authority (AAWSA, 2020). However, supply reliability is uneven, especially in the limits, where residents often resort to wells, boreholes, or private water vendors (UNICEF Ethiopia, 2021). The partial industrial expansion has raised concerns about potential groundwater contamination, prompting calls from environmental authorities for harsher regulatory oversight (Addis Ababa Environmental Protection Authority, 2022).

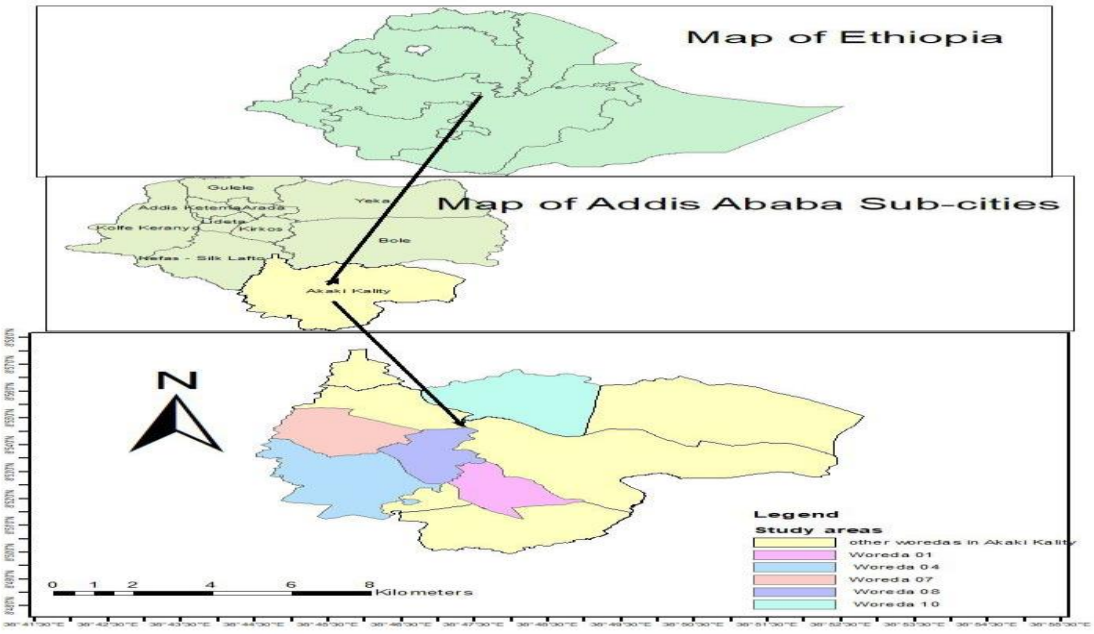

Figure 7: Map of the study area

3.2. Study design and sample size determination

A cross-sectional study design was employed in this study from November 2024 to June 2025. The sample size was determined using the formula provided by Thrusfield (2007) at a 95% confidence level, and a significance level of $p<0.05$ was set for both animal and human samples. An expected prevalence of 18.6% reported by Manyazewal (2018) was considered for calves, while a prevalence of 9% from Manyazewal (2017) was used for the study involving humans.

$$n = \frac{(1.96)^2 \, Pexp \, (1 - Pexp)}{d^2}$$

Where: n = required sample size; Pex*p* = expected prevalence; and d = desired absolute precision. So the calculated sample size can be:

$$n = \frac{(1.96)^2 \times 0.186(1-0.186)}{(0.05)^2} = 232 \text{ for calves and}$$

$$n = \frac{(1.96)^2 \times 0.09(1-0.09)}{(0.05)^2} = 124 \text{ for children's and}$$

Accordingly, 232 calves and 124 humans and 60 water samples (20 each from Akaki, Legedadi, and Gefersa rivers) were chosen based on similar waterborne protozoa studies (Robertson *et al.*, 2006; Castro-Hermida *et al.*, 2010), and aligned with WHO and USEPA guidelines for microbial water quality monitoring.

3.3. Study Population and Sampling Technique

3.3.1 Selection of study area

Akaki Kality Sub-City, specifically focusing on five districts-07, 01, 08, 04, and 10 was purposively considering several factors such as densely populated urban area with numerous water sources, The inclusion of the Woreda Health Centers of Kality and Gelan**,** collected water samples from various locations along the rivers (Akaki, Legedadi, and Gefersa), diverse population, with both human and animal residents, industrial activities in the region are a known source of environmental pollution, including contamination of water sources with chemical and biological agents, Diversity of Water Sources.

3.3.2. Study in the Calves population

Farms were selected using a simple random sampling method, while calves under one year of age were chosen purposively. This approach was applied due to the relatively small number of individual calves within the selected districts of the sub-city. By using purposive sampling, the study focused on areas where calves were known to be present, helping to ensure that the sample

accurately reflected the target population (Table 4).

Table 4: Calf population in the study districts

District	**Total No. of calf in the district**	**No. of calf sampled**
07	226	68
01	45	26
08	51	22
04	142	81
10	320	35
Total	**784**	**232**

3.3.3. Study involving children

A total of 124 children participated in the study, based on the estimated sample size for human subjects. This number was evenly distributed between two health centers located across the five woredas of Akaki Kality Sub-City (Table 5). Participants were selected using a stratified simple random sampling method. In addition to children visiting the health centers, the study also included children under the age of five from dairy farm communities, specifically, the children of farm owners and workers from the selected farms. For each child, one stool sample was collected along with questionnaire data to help identify possible risk factors for cryptosporidiosis. If consent to participate was not granted, the next eligible child was selected as a substitute (Annex 8).

Table 5: Study Children's population in the study Area

Name of health center	**District**	**Total children in the study district**	**No of children sampled**
Kality	07	112	36
	01	53	17
	08	27	9
Gelan	04	73	48
	10	32	14

3.3.4. Study involving drinking water sources

In this study, 60 water samples were collected from three rivers (Akaki, Legedadi, and Gefersa), with 20 samples per river to ensure equal spatial representation. which recommend stratified sampling to capture variability. Sampling was further distributed at 100-meter intervals and repeated twice daily to account for temporal changes due to human and environmental factors such as human activity patterns, temperature fluctuations, or natural river dynamics.

3.4. Sample collection and transportation

3.4.1. Calves fecal sample collection

Around 2 grams of faecal material were collected directly from the rectum of each calf using sterile gloves (Annex 3). The samples were then placed into sterile stool containers and immediately transported in a cold chain, using an ice box, to the Veterinary Parasitology Laboratory at the College of Veterinary Medicine and Agriculture, Addis Ababa University.

3.4.2. Children sample collection and transportation

Stool samples, each weighing about 2 grams, were collected from children under the age of five by trained laboratory technicians at the health centers, with help from the children's parents or guardians during the collection process (Annex 3). After collection, the samples were carefully transported to the College of Veterinary Medicine and Agriculture at Addis Ababa University for laboratory analysis.

3.4.3. Drinking water sample collection and transportation

Water samples were gathered from selected river sites by collecting large volumes usually around 10 liters using clean, sterile containers to ensure the samples remained free from contamination (Annex 6). Sampling points were established at 100-meter intervals along the rivers Akaki, Legedadi, and Gefersa. At each point, water was collected twice daily, once in the morning and once in the evening, to account for temporal variations, and transported to the Addis Ababa University College of Veterinary and Agriculture.

3.5. Study Methodology

3.5.1. Laboratory examination

To detect *Cryptosporidium* oocysts in fecal samples, a qualitative microscopic technique was employed. The diagnostic process involved the use of the modified acid-fast Ziehl-Neelsen staining method, following the concentration of oocysts using the formalin-ether concentration technique and centrifugal sedimentation. After concentration, thin smears were prepared from the sediment to distinguish oocysts from artifacts and other cells (Annex 4). Microscopy was then used to examine the smears for the presence of *Cryptosporidium* oocysts. These oocysts take up the acid-fast stain, appearing pink to red against a blue background. Known positive slides were used as controls to validate the staining and identification process.

Modified Acid-Fast Ziehl-Neelsen Staining Procedure

For fecal samples, approximately 2 grams of stool were mixed with 800 µL of distilled water and centrifuged to concentrate the oocysts (Annex 3). A thin smear (about 2 cm × 1 cm) was prepared from the sediment and allowed to air-dry. The smear was fixed with absolute methanol for 3 minutes, stained with 3% carbol-fuchsin for 15–20 minutes, decolorized using 3% acid-alcohol for 15–20 seconds, and counterstained with 0.1% methylene blue for 30–60 seconds. The slides were then examined under oil immersion (100× objective). *Cryptosporidium* oocysts were identified as pink to deep red spherical structures (4–6 µm in diameter), often with visible internal

sporozoites and a pale center. This staining also helped differentiate oocysts from uniformly red yeast cells (Annex 5).

For water samples, particulate matter was first concentrated by filtration, followed by centrifugation at 3,500 rpm for 10 minutes. The resulting pellet was resuspended in approximately 1 mL of distilled water. A drop (10–20 µL) of the suspension was placed on a slide, air-dried, and subjected to staining. The staining steps included 5 minutes with carbol-fuchsin, 1–2 minutes with acid-alcohol, and 1–2 minutes with methylene blue, with distilled water rinses between steps. Under oil immersion at 100× magnification, oocysts appeared as red or pink spherical structures measuring 4–6 µm, distinguished by visible sporozoites set against a blue background (Annex 7).

3.5.2. Questionnaire data

Beside sample collection, structured questionnaires were used to gather information on possible risk factors for cryptosporidiosis. For calves, the questionnaire included details about the production system, herd size, cattle breed, age, and body condition. It also covered the cleanliness of the pens, sources of drinking water, methods of farm waste disposal, presence of diarrhea, availability of calving pens, and how colostrum was given (Annex 1).

For human participants, the questionnaire gathered socio-demographic data including age, sex, parents' educational level, and occupation (e.g., farm owners, attendants, guards) and exposure risk factors (Annex 2).

3.6. Statistical analysis

All collected data were first entered into Microsoft Excel and then imported into R Studio for analysis. Descriptive statistics were used to summarize demographic, environmental, and clinical information, with results displayed through tables and graphs. To explore relationships between potential risk factors and *Cryptosporidium* infection, the Pearson Chi-square test was applied

initially. This was followed by a two-step logistic regression approach. First, univariable logistic regression identified which individual risk factors were significant. Variables with a p-value below 0.25 and no signs of multicollinearity were then included in a multivariable logistic regression model to determine the independent predictors of infection. Adjusted odds ratios (AORs) and 95% confidence intervals were calculated to measure the strength of these associations. Statistical significance was set at a p-value less than 0.05.

3.7. Ethical Considerations

Ethical approval for the study involving human participants was granted by the Ethical Review Board of the Aklilu Lemma Institute of Pathobiology Institutional Research Ethics Review Committee (ALIPB-IRERC). Written informed consent was obtained from all participants at the time of sample collection. They were assured that their samples and records would only be accessed by authorized personnel, that their personal information would remain strictly confidential, and that they could withdraw their consent at any point without any consequences. For the animal component of the study, ethical clearance was secured from the Ethical Review Committee of the University of Addis Ababa College of Veterinary Medicine. Before collecting samples and data, the purpose of the study was clearly explained to farm owners, and their permission was obtained.

4. RESULTS

4.1. Prevalence, Risk factors and Burden of Cryptosporidiosis in Calves

4.1.1. Prevalence and risk factors of Cryptosporidiosis in Calves

In this study, Cryptosporidiosis was found in 13.4% of calves, with 31 out of 232 fecal samples testing positive using the modified Ziehl-Neelsen staining technique. The infection was present in all five of the selected woredas. The highest prevalence was observed in Woreda 07 (19%; 13/68) (Figure 8).

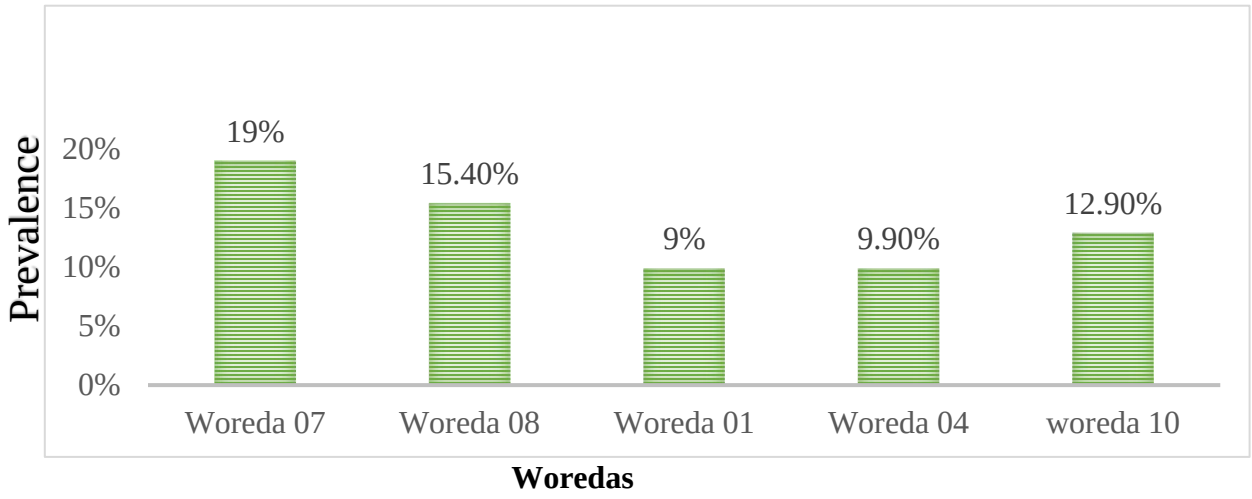

Figure 8: Prevalence of Cryptosporidiosis in calves by woreda

Out of the 232 calves examined, the prevalence of *Cryptosporidium* infection showed some variation across age categories. Calves younger than 3 months had a prevalence of 13.7%, those aged 3 to 6 months had a slightly higher rate of 15.6%, while calves aged 6 to 12 months had a lower prevalence of 9.8%. However, these variations were not statistically significant ($\chi^2 = 0.692$, $p = 0.872$). When analyzed by sex, infection rates were 13.8% in females and 12.7% in males, but this difference was also not significant ($\chi^2 = 0.999$, $p = 0.743$).

Regarding herd size, prevalence was 10.5% in large herds (more than 50 animals), 15.6% in medium-sized herds (10–50 animals), and 14.5% in small herds (fewer than 10 animals).

Although medium-sized herds showed a relatively higher prevalence, the variation among herd sizes was not statistically significant ($\chi^2 = 0.674$, $p = 0.143$).

In contrast, infection prevalence varied considerably across production systems: 12.3% in intensive systems, 12.8% in semi-intensive systems, and a markedly higher 50% in extensive systems. This difference was statistically significant, indicating that calves raised under extensive management systems were more likely to be infected ($\chi^2 = 0.040$, $p = 0.005$) (see Table 6).

Table 6: The prevalence of Cryptosporidiosis in calves by age, sex, herd size, and production system (N=232).

Variables	**Group**	**No. examined**	**No. Positive**	**Prevalence (%)**	χ^2	**P-value**
Age	<3 months	117	16	13.7	0.692	
	3-6months	64	10	15.6		0.872
	6-12 months	51	5	9.8		0.448
Sex	Female	130	18	13.8	0.999	
	Male	102	13	12.7		0.743
Herd size	Large (>50)	76	8	10.5	0.674	0.143
	Medium (10-50)	32	5	15.6		0.987
	Small (<10)	124	18	14.5		
Production	Intensive	187	23	12.3	0.040	
system	Semi-intensive	39	5	12.8		0.987
	Extensive	6	3	50		0.005*
Total		**232**	**31**	**13.4**		

*significant difference

The study examined risk factors linked to cryptosporidiosis in calves using a two-step analysis. Initially, univariable logistic regression was applied to explore possible connections between each individual risk factor and infection rates (Table 7). Next, a multivariable logistic regression was conducted to assess the impact of the significant factors while adjusting for other variables, with the findings summarized in Table 8.

Findings from the univariable analysis revealed that calves raised under extensive production systems exhibited a significantly higher infection rate (50%) compared to those in intensive systems, suggesting that limited management and greater environmental exposure may contribute to increased transmission ($p = 0.005$). Body condition also emerged as a significant predictor: calves in good physical condition showed a markedly lower prevalence (5.6%) than those in poor or moderate condition ($p = 0.007$), indicating that healthier animals may be more resilient to infection. Furthermore, the presence of a dedicated calving pen was associated with reduced risk; farms equipped with calving pens had lower infection rates (8.3%) compared to those without (20%) ($p = 0.043$).

Other variables including age group, sex, herd size, breed, fecal consistency, source of drinking water, pen hygiene, and the method of colostrum feeding did not show statistically significant associations with Cryptosporidiosis prevalence ($p > 0.05$) (Table 7).

Table 7: Univariate regression analysis of the association between *Cryptosporidium* infection and the assumed risk factors in calves (n=232)

Risk factors	Group	No. examined	Prevalence (%)	χ^2	95% CI	P-value
Age	<3 months	117	13.7	0.692		
	3-6months	64	15.6		0.747-3.960	0.872
	6-12 months	51	9.8		0.434-2.502	0.448
Sex	Female	130	13.8	0.999		
	Male	102	12.7		1.072-3.335	0.743
Herd size	Large (>50)	76	10.5	0.674	2.189-13.178	0.143
	Medium (10-50)	32	15.6		-2289-2251	0.987
	Small (<10)	124	14.5			
Production	Intensive	187	12.3	0.040		
system	Semi-intensive	39	12.8		-2288-2252	0.987
	Extensive	6	50		3.904-38.598	0.005*
Breed type	Exotic	189	12.7	0.720		
	Cross	35	14.3		-2251-98765	0.987
	Local	8	25		0.187-2.82	0.771
Body condition	Poor	123	13.8	0.003		
	Medium	38	26.3		-2252-67534	0.987
	Good	71	5.6		0.152-1.41	0.007*
Fecal consistency	Diarrheic	66	18.2	0.281		
	Non-diarrheic	166	11.4		0.948-2.643	0.314
Source of drinking water	River	46	19.6	0.690		
	Pipe	186	11.8		0.694-8.217	0.767
Cleanness of the pen	Unclean	94	17	0.055		
	Clean	138	10.9		0.796-3.923	0.580
Method of colostrum feeding	Suckling	142	9.9	0.126		
	Hand feeding	90	18.8		2.031-6.283	0.287
Calving pen	Absent	100	20	0.042		
	Present	132	8.3		0.177-3.415	0.043*
Total		**232**	**13.4**			

No. Exam= examined number, CI= Confidence Interval, *=significant difference.

The multivariable logistic regression analysis exposed three key factors significantly associated with Cryptosporidium infection in calves: the type of production system, the calf's body condition, and whether a calving pen was available (Table 8). In particular, calves raised under extensive production systems were found to be about 17 times more likely to contract the infection compared to those in intensive or semi-intensive systems (p = 0.005, OR = 17.45, 95% CI: 3.90–38.60).

Table 8: The major risk factors of Cryptosporidiosis in calves based on multivariable logistic regression analyses (N=232)

Risk factors	**Group**	**Prevalence (%)**	χ^2	**Adjusted OR**		**95% CI**
				OR	**95% CI**	
Production system	Intensive	12.3	0.040			
	Semi-intensive	12.8		0.00	-2288-2252	0.987
	Extensive	50		17.45	3.904-38.598	0.005*
Body condition	Poor	13.8	0.003			
	Medium	26.3		8540.00	-2252-67534	0.987
	Good	5.6		0.54	0.152-1.41	0.007*
Calving pen	Absent	20	0.042			
	Present	8.3		0.42	0.177-3.415	0.043*
Total		**13.4**				

OR=Odds Ratio χ2=Chi-square CI= Confidence Interval, *=significant difference

4.1.2. Burden of infection in Calves

Microscopic analysis showed varying levels of *Cryptosporidium* oocyst burden among calves, depending on their location, age, and sex. The oocyst load was classified into low, moderate, and high based on the number of oocysts observed per microscopic field. Among the 31 positive samples, the highest proportion of high-intensity infections was recorded in Woreda 4 (5 out of 8 cases; 62.5%) and Woreda 8 (3 out of 5 cases; 60%). Woreda 7 reported the greatest number

of total positive cases (11), although the oocyst burden in this area was more evenly distributed across all burden categories (Figure 9).

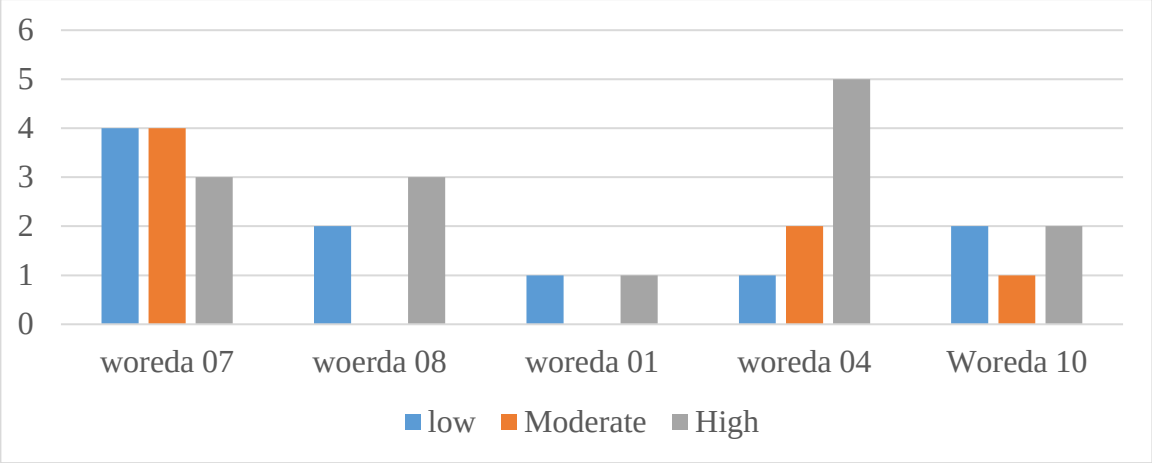

Figure 9: Burden of *Cryptosporidium* infection in Calves in the study Woredas

Among the various age groups examined, calves under 3 months of age showed the highest oocyst burden, with 11 out of 16 positive cases (68.8%) presenting a high level of oocyst shedding (Figure 10).

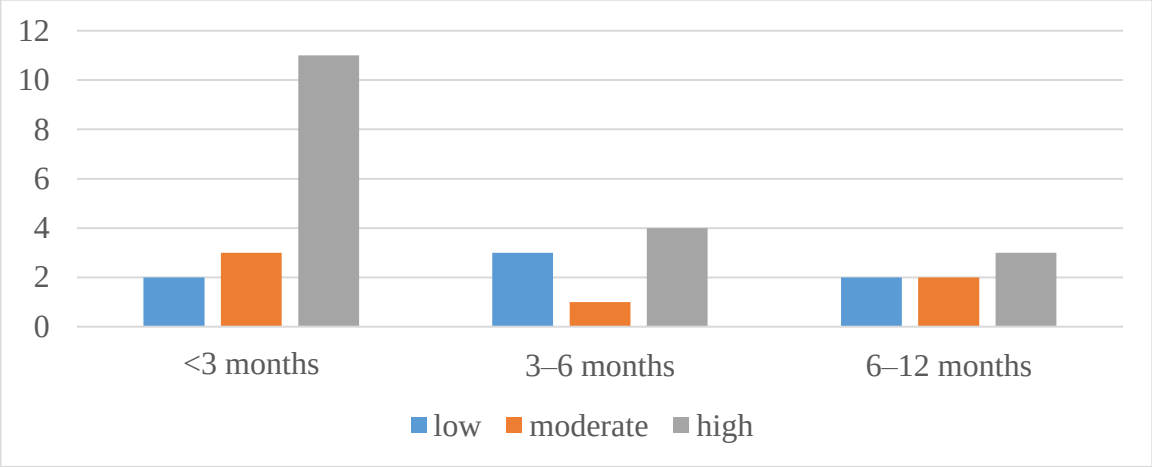

Figure 10: Burden of infection in calves by age category

In a study examining *Cryptosporidium* infection rates, it was noted that female calves exhibited a higher burden of infection compared to male calves, as illustrated in (Figure 11).

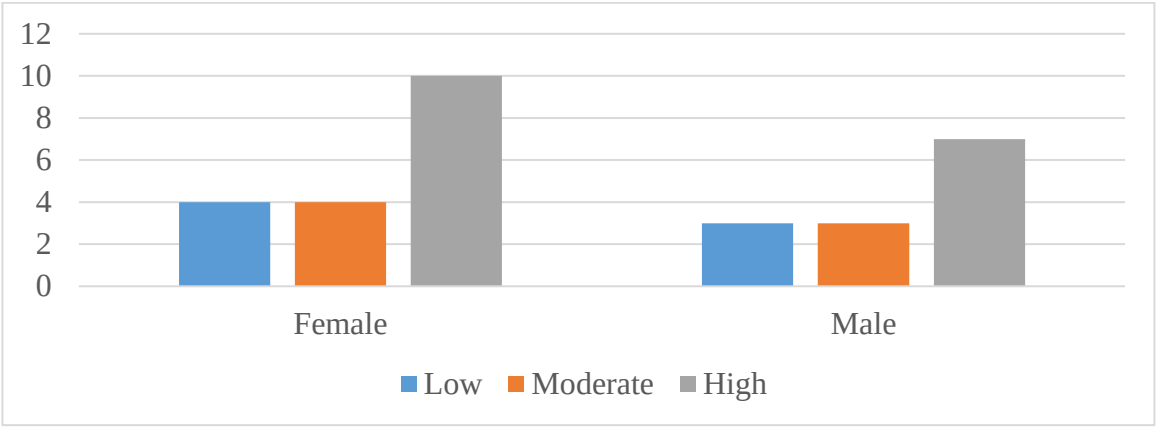

Figure 11: Burden of *Cryptosporidium* Infection in calves by sex

4.2. Prevalence, risk factors and burden of Cryptosporidiosis in children

4.2.1. Prevalence, risk factors of Cryptosporidiosis in children

Out of 124 stool samples collected from children visiting health centers in Akaki Kality Sub-City, microscopic analysis identified oocysts in 9 cases, reflecting an overall prevalence of 7.3%. At Kality Health Center, 6 of the 62 children screened (9.7%) tested positive, while Gelan Health Center observed 3 positive cases (4.8%) from an equal number of samples (Figure 12).

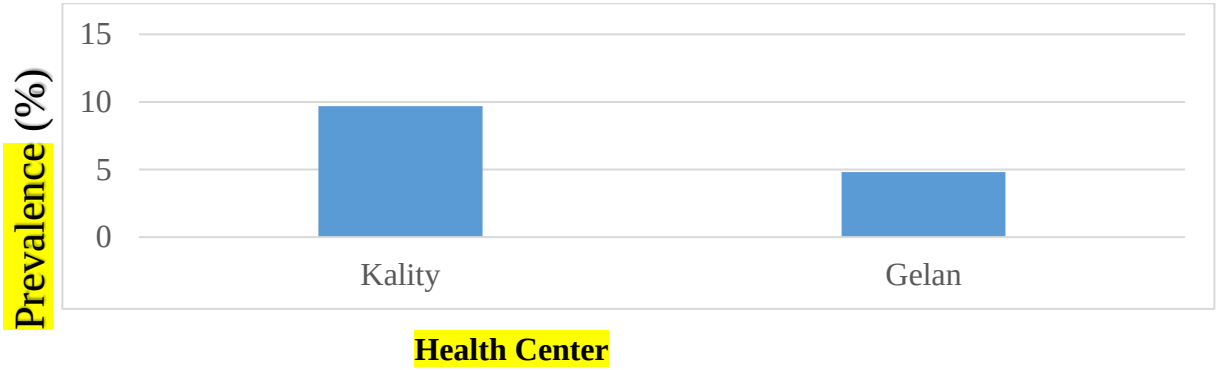

Figure 12: Prevalence of in children by health centers

The distribution of positive cases also differed across woredas, with Woreda 07 observed the highest number 5 positive cases out of 36 children tested, representing a prevalence of 13.9% (Figure 13).

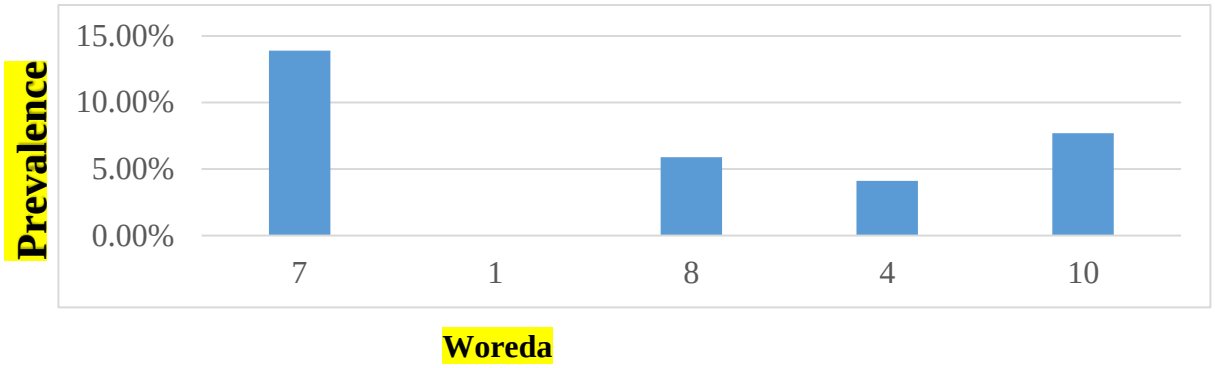

Figure 13: Prevalence of in children in different woredas

In this study, neither age nor sex showed a significant association with the prevalence of Cryptosporidiosis ($p > 0.05$), despite a slightly higher occurrence in younger children and females. However, parental literacy and contact with animals emerged as significant risk factors. Children whose parents were illiterate had a notably higher infection rate (23.5%) compared to those with literate parents (4.7%) ($p = 0.008$) (Table 9).

Table 9: The prevalence of Cryptosporidiosis in children by demographic characters

Variables	**Label**	**No. Examined**	**No. Positive**	**Prevalence (%)**	χ^2	**P-value**
Age	less than one year	69	5	7.2	0.896	
	1-3 years,	39	4	10.6		0.332
	3-5 year	16	0	0		0.685
Sex	Male	62	4	6.5	0.158	
	Female	62	5	8.1		0.672
Parent	Illiterate	17	4	23.5	0.0003	
education	Literate	107	5	4.7		0.008*

In this study, children experiencing diarrhea exhibited a higher prevalence of *Cryptosporidium* (24%) compared to those without diarrhea (3%); however, this association was not statistically significant (p = 0.438). A significant risk factor identified was animal contact, which showed a greater prevalence among exposed children (17.9%) versus non-exposed ones (2.4%) with a p-value of 0.049. Particularly, there were no infections detected in children whose parents practiced regular handwashing, whereas those who did not had a prevalence rate of 22% (p = 0.092). Additionally, children consuming river water had a prevalence of 9.8%, which was higher compared to 5.5% among those using piped water, although this finding was also not statistically significant (Table 10).

Table 10: The prevalence of Cryptosporidiosis in children based on different risk factors in the study area (n=124).

Variables	**Label**	**No. Examined**	**No. Positive**	**Prevalence (%)**	χ^2	**P-value**
Presence of diarrhea	No	99	3	3	0.929	
	Yes	25	6	24		0.438
Contact with animals	No	85	2	2.4	0.0095	
	yes	39	7	17.9		0.049*
Parent hand washing	Yes	83	0	0	0.079	
	No	41	9	22	0.764	0.092
Drinking water source	River	51	5	9.8		
	Pipe	73	4	5.5		0.763

*significant difference

4.2.2. Burden of Cryptosporidiosis in Children

Out of the nine confirmed cases, six (66.7%) were categorized as low burden and three (33.3%) as moderate burden. No high-burden cases were detected. Both health centers reported an equal

number of low-burden cases (three each), while Kality had a slightly higher number of moderate cases (two) compared to Gelan (one) (Figure 14).

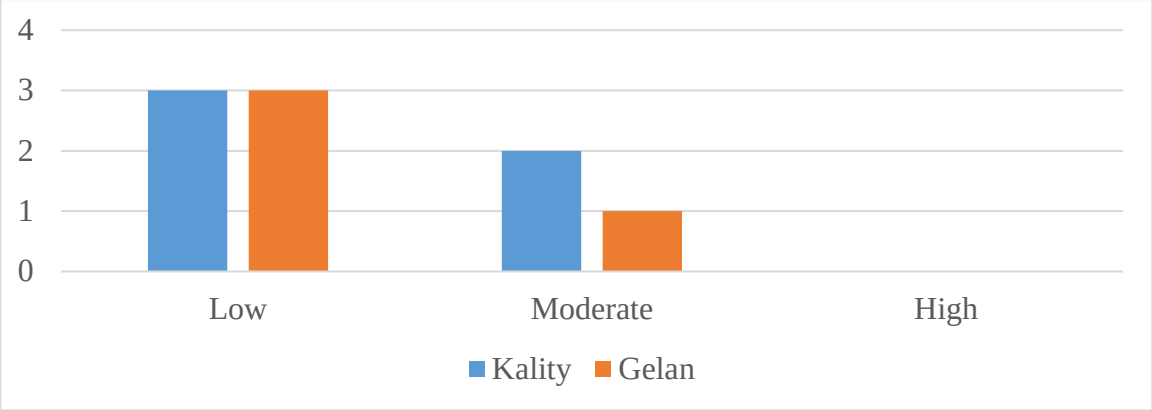

Figure 14: Burden of Cryptosporidiosis in Children by health Center

The burden of Cryptosporidiosis among children differed across the woredas in Akaki Kality Sub-City. Woreda 07 recorded the highest number of cases, with five children affected three with low and two with moderate infection levels. Woredas 04 and 08 each observed one case with a low burden, while Woreda 10 had one case with a moderate burden. No cases were detected in Woreda 01 (Figure 15).

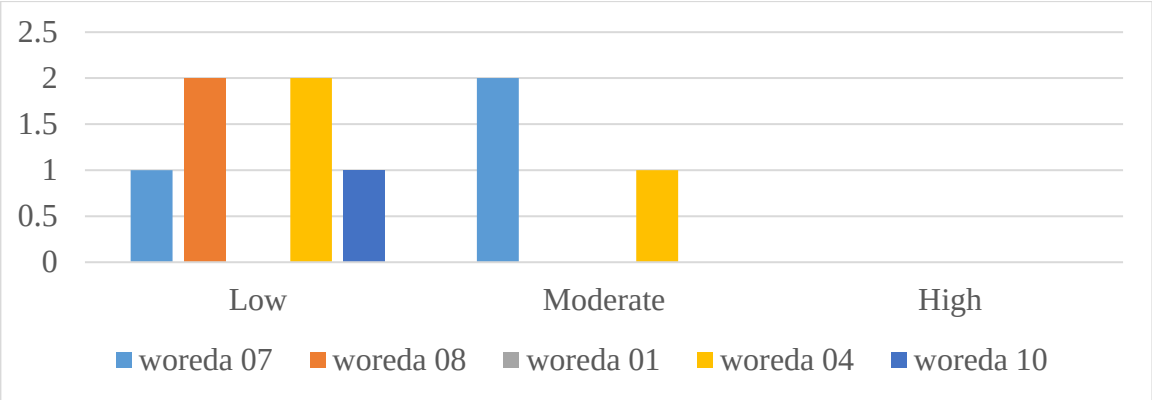

Figure 15: Burden of Cryptosporidiosis in Children by Woreda

Children under one year were the most affected, accounting for all moderate cases and half of the low-burden cases (66.7% total). The remaining low-burden cases were in children aged 1–3 years, while no cases were reported in the 3–5-year age group (figure 16).

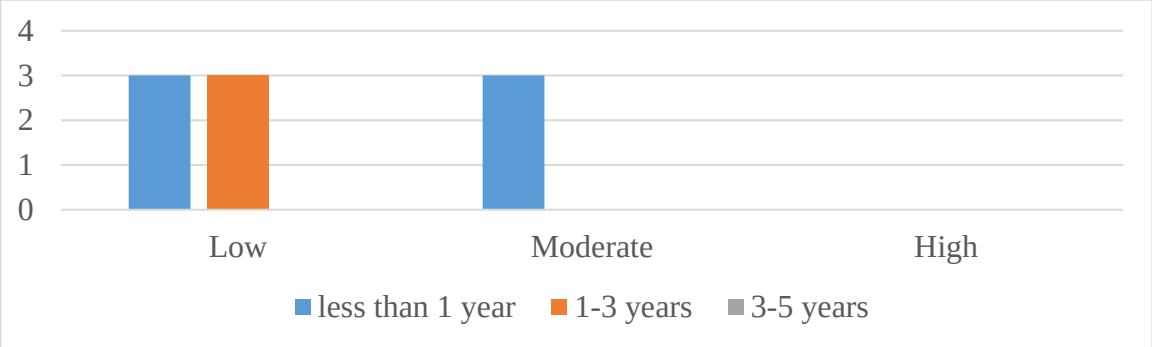

Figure 16: Burden of Cryptosporidiosis in Children by Age

Among the different sex observed, the highest burden of *Cryptosporidium* infection was observed in females 55.6% and males 44.4% (Figure 17). No high-burden cases were reported in either group.

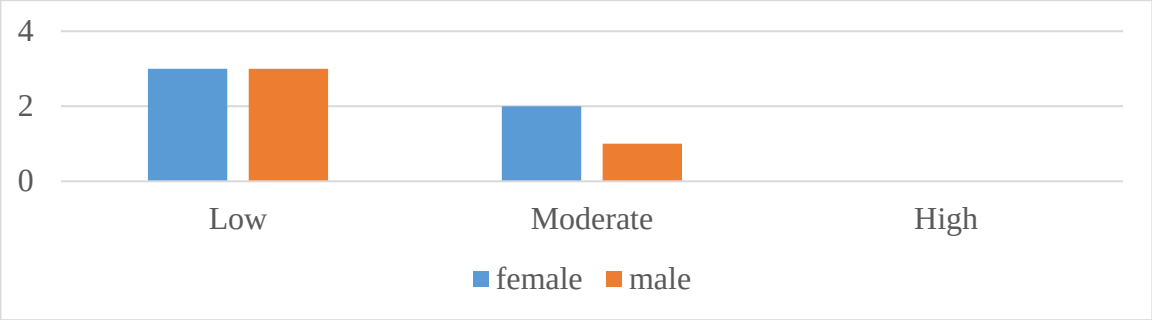

Figure 17: Burden of Cryptosporidiosis in Children by sex

4.3. Prevalence and burden of Cryptosporidiosis in drinking water sources

4.3.1. Prevalence of Cryptosporidiosis in Drinking water sources

In this study, the overall prevalence of *Cryptosporidium* in drinking water sources was 60%, determined using the Modified Ziehl-Neelsen acid-fast staining method. All water samples from Akaki tested positive, revealing widespread contamination in that area. In contrast, every sample from Legedadi was negative, indicating no detectable Cryptosporidium presence. Meanwhile, 80% of the samples from Gefersa were positive, demonstrating varying degrees of contamination among the different water sources (Figure18).

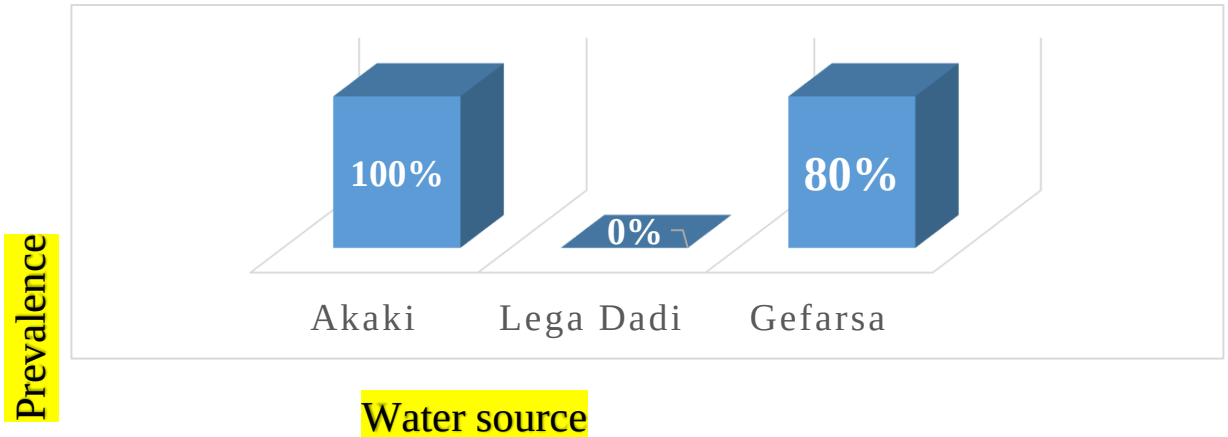

Figure 18: Prevalence of Cryptosporidiosis in drinking water sources.

4.3.2. Burden of Cryptosporidiosis in drinking water Sources

Out of 60 drinking water samples observed, 60% tested positive for oocysts, highlighting a significant level of environmental contamination. Akaki Kality showed the highest contamination, with all samples positive and 55% exhibiting a high burden. Gefersa followed with 80% positive samples, including high, moderate, and low burden levels. In contrast, no oocysts were detected in samples from Lege Dadi, indicating the absence of contamination in that water sources (Figure 19).

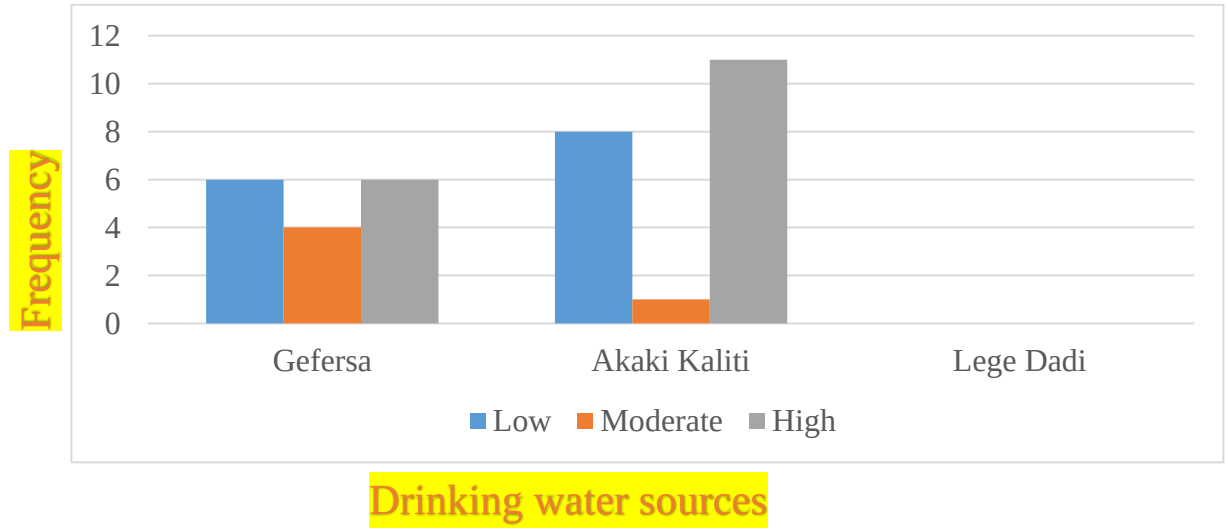

Figure 19: Burden of Cryptosporidiosis in drinking water sources

5. DISCUSSION

5.1. Prevalence, risk factors, and burden of Cryptosporidiosis in Calves

This study detected *Cryptosporidium* infection in 13.4% of calves in the area, a rate that is quite similar to what has been reported in other regions of Ethiopia such as 13.6% reported in Gondar (Kassa *et al.*, 2017) and 12.9% in the central highlands (Tulu *et al.*, 2023). However, this rate is notably lower than the 28.7% prevalence documented in Jimma by Alemayehu et al. (2021), highlighting regional differences likely influenced by variations in environmental conditions, management practices, and sanitation. Additionally, methodological differences, particularly the reliance on acid-fast microscopy with its lower sensitivity compared to PCR techniques, may partly explain these discrepancies (Widmer *et al.*, 2020).

Cryptosporidiosis was detected in all sampled woredas, with the highest rate (19%) observed in Woreda 07, suggesting that differences in farm hygiene, calf management, and environmental contamination levels might influence spatial variation. Calves between 3 and 6 months old had a slightly higher infection rate (15.6%) compared to younger or older calves, but this difference wasn't statistically meaningful. This pattern matches earlier studies showing that young calves, particularly in their first few months, are more susceptible to infection because their immune systems are still developing and they are more exposed to contaminated surroundings (Santín, 2020).

Sex did not significantly influence infection rates, which corroborates earlier studies from Ethiopia and Kenya (Zeleke *et al.*, 2022; Kiiru *et al.*, 2019). Female calves had a marginally higher prevalence (13.8%) compared to males (12.7%), but the difference was not statistically meaningful. Among the risk factors examined, production system, body condition, and the availability of a calving pen emerged as significant predictors of infection in the multivariable model. Calves reared in extensive production systems had the highest infection rate (50%), a statistically significant finding ($p = 0.005$). This supports observations by Gebretsadik *et al.* (2021) who noted that extensive systems often lack adequate biosecurity measures, leading to

increased contact with contaminated soil and water. Similarly, Alemu *et al.* (2020) emphasized that the absence of controlled housing in such systems raises calves' environmental exposure to oocysts, reinforcing the present study's results.

Body condition was another important factor, with calves in good condition showing much lower infection rates (5.6%) compared to those in medium condition (26.3%, $p = 0.007$). Poor nutrition can weaken the immune system, increasing susceptibility to infection (Thomson *et al.*, 2023). The presence of a calving pen also significantly lowered infection risk ($p = 0.043$). Calves born in designated pens were less likely to be infected, aligning with One Health principles that stress environmental hygiene to reduce zoonotic disease transmission (Grace *et al.*, 2022). Calving pens likely limit early exposure to contaminated surroundings, reducing oocyst ingestion during the vulnerable neonatal period.

Microscopic assessment of infection burden revealed notable variation across locations, age groups, and sex. Oocyst shedding was categorized as low, moderate, or high based on the number of oocysts observed per microscopic field, providing a semi-quantitative measure of infection intensity.

Geographically, Woreda 4 had the highest proportion of calves with heavy oocyst loads (62.5%), followed by Woreda 8 (60%). Although Woreda 7 had the greatest total number of positive cases (11), the distribution of burden there was more balanced. These differences likely reflect variability in farm hygiene, management, and environmental contamination levels.

Age-wise, calves under three months had the highest burden, with 68.8% (11 out of 16) classified as high shedders. This supports previous studies showing that neonatal calves are more prone to heavy infections due to their immature immune systems and greater environmental exposure early in life (Thomson *et al.*, 2022). Such heavy shedding in young calves underscores their key role in contaminating the environment and maintaining transmission within herds.

Regarding sex, female calves had a slightly higher proportion of high oocyst shedding (55.6%) compared to males (53.8%), but this difference was minimal and not statistically significant, echoing findings from other epidemiological studies where infection intensity was more influenced by management than biological sex (García-Rubio *et al.,* 2019).

Overall, these findings reinforce that young calves raised in environments with suboptimal hygiene are at greater risk for heavy *Cryptosporidium* infections. High parasite loads in neonates are particularly concerning because they contribute substantially to environmental contamination and ongoing transmission cycles. Moreover, heavy infections are linked to more severe clinical outcomes, increasing morbidity and mortality among neonatal ruminants (García-Rubio *et al.*, 2019). This highlights the urgent need for targeted interventions focusing on neonatal care, improved biosecurity, and enhanced hygiene measures with in a One Health framework.

5.2. Prevalence, risk factors, and burden of Cryptosporidiosis in children

This study found a 7.3% prevalence of *Cryptosporidium* infection among children attending health centers in Akaki Kality Sub-City, Addis Ababa. This rate aligns with findings from similar urban areas in sub-Saharan Africa, where prevalence typically ranges from 5% to 15% (Zhou *et al.,* 2022; Ramo *et al.,* 2021). The slightly higher prevalence at Kality Health Center (9.7%) compared to Gelan Health Center (4.8%) may reflect differences in local environmental contamination or healthcare-seeking behaviors.

Geographically, Woreda 07 showed the highest infection rate at 13.9%, supporting previous studies that demonstrate how prevalence often clusters due to variations in water sanitation, animal contact, and hygiene practices (Thomson *et al.*, 2022). Conversely, no cases were detected in Woreda 01, possibly indicating better sanitation or reduced environmental exposure in that area.

Age-wise, children aged 1–3 years exhibited the highest prevalence (10.6%), consistent with existing literature that highlights children increased vulnerability due to developing immunity

and more frequent exploratory behaviors (García-Rubio *et al.,* 2019). Although differences by age and sex were not statistically significant, the slightly higher prevalence in females (8.1%) compared to males (6.5%) aligns with some regional studies (Adamu *et al.,* 2020), though the reasons behind this disparity remain unclear and deserve further research.

Regarding risk factors, children of illiterate parents had a significantly higher infection rate (23.5%, $p = 0.008$), suggesting that lower parental education may limit awareness and implementation of preventive measures (WHO, 2023). Additionally, children with animal contact showed a significantly increased prevalence (17.9%, $p = 0.049$), underscoring the zoonotic nature of transmission in urban settings (Xiao & Feng, 2017). These findings are consistent with the One Health framework, which highlights the interconnection between human, animal, and environmental health (FAO, OIE & WHO, 2021).

Although not statistically significant, children experiencing diarrhea had a notably higher prevalence (24%), and those whose parents did not regularly practice handwashing showed a 22% infection rate. These observations reinforce the critical role of hygiene and suggest that interventions focused on hand hygiene and diarrhea management could substantially reduce infection risk.

Microscopic assessment of infection burden revealed that two-thirds (66.7%) of positive children had a low parasite burden, while one-third (33.3%) had a moderate burden. No high-burden cases were detected, possibly indicating mild infections or early-stage oocyst shedding, which is consistent with pediatric studies in similar low-income settings (Bertolini *et al.,* 2022). The distribution of burden was fairly balanced between Kality and Gelan Health Centers, with no significant clustering of severe cases. Woreda 07, which had the highest overall prevalence, also accounted for the majority of moderate-burden cases, reinforcing its identification as a transmission hotspot.

Age-specific analysis showed that all moderate-burden infections occurred in children under one-year-old, confirming that infants not only have greater susceptibility to infection but may also

shed heavier oocyst loads. This matches prior findings showing neonates and young infants bear the highest infection burden due to immature immune systems and closer environmental exposure (Thomson *et al.*, 2022).

Analysis by sex revealed no high-burden cases in either males or females, with a slightly higher proportion of moderate burden in females (40% vs. 25%). However, this small difference is likely due to sample size limitations and may be incidental.

Overall, these findings highlight the urgent need for targeted interventions focusing on young children, particularly those in high-risk woredas, with animal contact, and from families with lower educational attainment. Enhancing hygiene education, improving water quality, and integrating zoonotic disease control efforts within the One Health.

5.3. Prevalence and burden of Cryptosporidiosis in drinking water Sources

This study revealed a notably high prevalence of *Cryptosporidium* oocysts in drinking water sources within Akaki Kality Sub-City, Addis Ababa. Using the modified Ziehl-Neelsen acid-fast staining technique, 60% (36 out of 60) of the water samples tested positive for oocysts. Samples were evenly collected from three main water sources Akaki, Legedadi, and Gefersa with 20 samples taken from each location. Extremely, all samples from the Akaki source (20/20; 100%) were contaminated, indicating severe pollution. In contrast, none of the samples from Legedadi (20/20; 0%) showed presence of oocysts, suggesting effective protection of this water source. Gefersa also demonstrated a high contamination rate, with 80% (16/20) of its samples testing positive.

This variation in contamination levels likely reflects differences in watershed management, human activities, and livestock presence near the water catchments. The heavy contamination in Akaki could be attributed to urban runoff and insufficient wastewater treatment, which have been previously linked to pollution from domestic and industrial discharges in the area (Gebrezgiabher *et al.*, 2021). The high oocyst presence in surface water sources like Gefersa aligns with earlier

research showing that open water systems are more vulnerable to protozoal contamination, especially during rainy seasons when runoff intensifies pathogen transport (Efstratiou *et al.*, 2017).

Beyond prevalence, the study also assessed the intensity of contamination using a semi-quantitative microscopy-based scoring system that categorized oocyst load into four groups: No burden, Low, Moderate, and High. Among the 36 positive samples, the level of contamination varied significantly across sources.

Akaki water samples not only had universal contamination but also the highest proportion of heavily contaminated samples, with 11 out of 20 (55%) classified as high burden. This poses a serious public health risk, as high oocyst loads in untreated or inadequately treated water increase the likelihood of outbreaks, particularly among vulnerable groups such as immunocompromised individuals (Chalmers & Katzer, 2013). The remainder of Akaki's positive samples included 9 with low oocyst burden and 1 with moderate levels.

Gefersa, while slightly less contaminated overall, still poses a health concern. Of its 16 positive samples, 6 were classified as high burden, 4 as moderate, and 6 as low, indicating sporadic but potentially significant contamination events likely driven by livestock grazing and human activity near the reservoir. Conversely, Legedadi's clean water source with no detected oocysts in any samples highlights the benefits of well-managed and protected water supplies.

These findings underscore the critical need for improved source water protection and enhanced monitoring alongside robust treatment protocols. *Cryptosporidium* oocysts are notably resistant to conventional chlorination and can persist in water supplies, making filtration and ultraviolet disinfection essential components of effective water safety plans (Ryan *et al.*, 2021). The elevated oocyst burden observed in Akaki and Gefersa points to a real risk of both zoonotic and human-to-human transmission via contaminated water, reinforcing the importance of a One Health approach that combines environmental management, veterinary oversight, and public health surveillance to tackle this challenge comprehensively.

6. CONCLUSION AND RECOMMENDATIONS

Using a One Health approach, this study assessed the prevalence, impact, and contributing risk factors of *Cryptosporidium* infection in calves, children under five, and drinking water sources in Akaki Kality Sub-City. Findings observed a 13.4% prevalence in calves, 7.3% in children, and 60% contamination in detected drinking water sources. Significant risk factors in calves identified include production systems, poor body condition, and absence of calving pens. In children, parental illiteracy and animal contact were key predictors. The highest infection burdens were found in young calves and infants under one year. Children who have had contact history with animals have higher prevalence than those which didn't have contact with animals. These findings highlight the zoonotic and waterborne transmission potential of Cryptosporidiosis and its public health importance in the study area.

Based on these findings, the following recommendations were proposed:

- Promote better feeding, hygiene, and the use of calving pens, especially in extensive systems.
- Raise awareness about hygiene and zoonotic transmission, especially among caregivers and farm workers.
- Improve water treatment, protect clean sources like Legedadi, and monitor high-risk sources like the Akaki River.
- Foster collaboration between animal, human, and environmental health sectors for early detection and response.
- Conduct molecular studies to identify local species and enhance diagnostic capacity.

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7. ANNEXES

ANNEX 1: Questionnaire Format for Calf-Related Data Collection

A. General Information on the Dairy Farm

1. Name of Farm: _____
2. Owner's Name: _____
3. Address: Kebele _____ House No. _____ Tel. No. _____

B. Location and Production System

4. Farm Location: ☐ Urban ☐ Peri-urban ☐ Rural

5. Production System: ☐ Intensive ☐ Semi-intensive ☐ Extensive

6. Herd Size: ☐ Small ☐ Medium ☐ Large

C. Water Source, Sanitation, and Housing

7. Presence of Calving Pen: ☐ Present ☐ Absent

8. Cleanliness of Pen: ☐ Clean ☐ Moderate ☐ Dirty

9. Source of Drinking Water: ☐ Well ☐ River ☐ Pipe

10. Wastewater Disposal Method: ☐ To a well ☐ To a river ☐ To grazing field

11. Type of Housing: ☐ Separate pen ☐ Together with cows ☐ Other (specify): _____

12. If separate pen, type: ☐ Individual ☐ Group

D. Calf-Specific Information

13. Calf Code No.: _____

14. Date of Birth: Day: _____ Month: _____ Year: _____ Age: _____

15. Sex: ☐ Male ☐ Female

16. Breed: ☐ Local ☐ Crossbred ☐ Exotic

17. Fecal Consistency: ☐ Diarrhea ☐ Soft ☐ Solid

ANNEX 2: Questionnaire Format for Human Participants (Children of Dairy Farm Households)

1. Participant Code No.: _____
2. Name: _____District: _____ Kebele: _____ Date: _____
3. Sex: ☐ Male ☐ Female
4. Age: _____
5. Father's Occupation: ☐ Farm Owner ☐ Farm Attendant ☐ Other (specify): _____
6. Father's Education Level: ☐ Illiterate ☐ Primary ☐ Secondary ☐ College/University
7. Hand Washing Habit: ☐ Good ☐ Poor
8. Fecal Consistency: ☐ Diarrhea ☐ Soft ☐ Solid
9. Household Wastewater Disposal: ☐ Open field ☐ Burn ☐ Other: _____
10. Presence of Household Animals: ☐ Yes ☐ No
11. Animal Types Present: ☐ Cattle ☐ Dog ☐ Cat ☐ All ☐ Other (specify): _____

ANNEX 3: Procedure for Fecal Sample Collection and Preparation

1. Wash hands thoroughly and wear new disposable gloves.
2. Remove and set aside the lid of the specimen container.
3. Prepare the collection container.
4. Collect stool directly, avoiding contamination with water or urine.
5. Use the spoon attached to the container lid to scoop from the most representative part of the stool (bloody, slimy, or watery sections preferred).
6. Transfer enough stool to the container to reach the "fill to here" line.
7. Secure the lid tightly and shake the container gently to mix.
8. Label the specimen clearly with: participant name, age, date, and time of collection using waterproof marker.
9. Place the container in a zip-lock bag and seal securely.
10. Store sample on ice or in a refrigerator immediately.

11. If not immediately transported:

- Room temperature: < 1 hour
- Refrigerator (4°C): 1–48 hours
- Freezer (–20°C): 2–60 day

ANNEX 4: Concentration Techniques and Reagents Used for Laboratory Examination

1. Formalin-Ether Concentration Technique

This technique is widely used in reference laboratories for the concurrent diagnosis of helminths and intestinal protozoa. It enhances detection sensitivity by concentrating parasitic elements through centrifugation. During this process, fecal debris partitions into the ether phase, while parasites sediment at the bottom.

Procedure:

1. Prepare the Sodium Acetate–Acetic Acid–Formalin (SAF) solution by dissolving 15 g of sodium acetate in 20 mL acetic acid and 40 mL of 40% formalin, diluted in 925 mL of distilled water.
2. Preserve ~1 g of fresh stool in 10 mL of SAF solution in a sealed, water-tight container.
3. Re-suspend the fixed sample and strain it through gauze layered over a funnel.
4. Collect the filtrate in a 15 mL centrifuge tube and centrifuge at 2,000 rpm for 1 minute.
5. Carefully discard the supernatant without disturbing the sediment.
6. Add 7 mL of 0.9% saline to the sediment, mix gently with a wooden applicator, then add 3 mL of ether.
7. Cap the tube tightly and shake vigorously for 30 seconds. Remove the stopper cautiously due to pressure build-up.
8. Centrifuge again at 2,000 rpm for 5 minutes.
9. After centrifugation, four layers should be visible. Discard the top three (ether, debris, and saline) layers.
10. Mix the sediment thoroughly and place a drop onto a slide.
11. Examine under a microscope using a 40× objective and confirm using 100× oil immersion for protozoa and oocysts.

2. Absolute Methanol

Absolute methanol is a flammable, colorless liquid used as a fixative in acid-fast staining techniques. It ensures optimal adhesion of fecal smears to slides and is a reagent-grade substance conforming to international standards of purity.

3. 3% Carbol-Fuchsin Solution

Carbol-fuchsin serves as the primary stain in the modified Ziehl-Neelsen technique, selectively staining acid-fast oocysts such as .

Preparation Procedure:

1. Weigh 3 g of basic fuchsine and dissolve in 100 mL absolute ethanol (Solution 1), warming at 60°C.
2. Separately dissolve 5 g phenol crystals in 50 mL distilled water (Solution 2).
3. Mix Solutions 1 and 2, then top up with distilled water to a final volume of 100 mL.
4. Filter using Whatman No. 1 paper and store in an amber bottle.
5. Label with reagent name, lot number, preparation and expiration dates, and storage conditions.

4. Acid-Alcohol (Decolorizer)

This solution decolorizes non-acid-fast elements during staining.

Preparation:

Mix 2 mL concentrated hydrochloric acid with 98 mL of 95% ethyl alcohol.

5. 0.1% Methylene Blue (Counterstain)

Methylene blue is used to provide contrast, staining background material blue to differentiate acid-fast oocysts.

Preparation Procedure:

1. Weigh 0.1 g methylene blue and dissolve in 100 mL distilled water.
2. Filter the solution and store in an amber glass bottle.
3. Label as per laboratory reagent standards.

6. Oil Immersion

Oil immersion microscopy is used to improve resolution when examining oocysts under high magnification. A drop of immersion oil is applied directly on the stained smear and examined using the 100× objective lens, enhancing the clarity of small structures like oocysts (4–6 µm in size).

ANNEX 5: Modified Ziehl-Neelsen (Acid-Fast) Staining Procedure

This staining method is essential for detecting acid-fast oocysts in fecal and environmental samples.

Staining Steps:

1. Prepare a thin stool smear directly or from concentrated sediment.
2. Allow the smear to air dry completely.
3. Fix the dried smear using absolute methanol for 3 minutes.
4. Stain the slide with 3% carbol-fuchsin for 15–20 minutes.
5. Rinse gently with tap water.
6. Decolorize using acid-alcohol (3%) for 15–20 seconds.
7. Rinse again with tap water.
8. Counterstain with 0.1% methylene blue for 30–60 seconds.
9. Apply a drop of immersion oil.
10. Examine under the microscope using 40× and 100× objectives.

ANNEX 6: Modified Procedure for Detection of Oocysts from Drinking Water Sources

1. Water Sampling and Filtration

- **Sample Collection:** Collect 10 liters of water from suspected contaminated sources using sterile containers.
- **Filtration:** Filter the collected water through appropriate membrane filters to capture particulate matter, including oocysts.

2. Centrifugation and Concentration

- **Eluate Processing:** Elute the retained material from the filter into a container.
- **Centrifugation:** Spin the eluate at 3,500 rpm for 10 minutes.
- **Supernatant Removal:** Carefully decant the supernatant without disturbing the pellet.

3. Slide Preparation

- **Pellet Resuspension:** Resuspend the pellet in ~1 mL of distilled water.
- **Application to Slide:** Place 10–20 µL of the suspension on a clean glass slide.
- **Air Drying:** Allow the sample to air dry completely before staining.

4. Acid-Fast Staining (Modified Ziehl-Neelsen)

- **Primary Stain:** Apply Carbol Fuchsin for 5 minutes.
- **Decolorization:** Rinse with acid-alcohol for 1–2 minutes.
- **Counterstain:** Apply Methylene Blue for 1–2 minutes.
- **Inter-step Rinsing:** Gently rinse with distilled water between steps.

5. Microscopic Examination

- **Microscope Use:** Examine the slide under 100x oil immersion.
- **Identification:** Identify oocysts as red-stained, spheroid bodies (4–6 µm) against a blue background.

6. Enumeration and Reporting

- **Counting:** Enumerate visible oocysts per slide.
- **Documentation:** Report the number of oocysts per volume of water sampled, along with relevant metadata.

ANNEX 7: Interpretation of Samples Stained Using the Modified Ziehl Neelsen Method and Microscopy grading was based on oocyst counts per high-power field (HPF).

The interpretation of stained fecal or environmental samples was based on the morphological and staining characteristics of oocysts under light microscopy at 100× oil immersion magnification.

A sample was considered **positive** for spp. if it demonstrated the following features:

- **Morphology:** Round to slightly oval shape
- **Color:** Bright pink to red staining with a **pale or clear center**
- **Size:** Approximately **4–6 µm** in diameter
- **Appearance:** Refractile oocysts with well-defined borders, distinguishable from background debris and non-acid-fast organisms such as yeasts
- **Number:** Presence of **at least one characteristic oocyst** per visual field was considered positive

Negative samples exhibited no acid-fast oocysts and were characterized by a uniformly blue background with no red- or pink-stained elements in the expected size range.

Microscopy grading was based on oocyst counts per high-power field (HPF)

Infection Intensity	**Oocysts per HPF**	**Grading Description**
Low	<5 oocysts per HPF	Sporadic detection
Moderate	5–10 oocysts per HPF	Easily detectable
High	>10 oocysts per HPF	Numerous oocysts visible

ANNEX 8: Written Consent Form

English Version

S/No.: _____

Card No.: _____

Full Name: _____

I, the undersigned, confirm that the details of the study titled:

"STUDY ON INFECTION IN CALVES, CHILDREN, AND DRINKING WATER SOURCES IN AKAKI KALITY, ADDIS ABABA",

being conducted in Akaki Kality Sub-City at _____ Health Center, have been clearly explained to me. I understand the study's purpose, potential benefits, possible risks, and the confidentiality measures being taken to protect my personal information. I have had the opportunity to ask questions, and I understand that I may withdraw from the study at any time without penalty or loss of benefits. I am also aware that my data would be anonymized using numerical codes rather than my name. I understand that I would not receive any direct personal benefit except the clinical services related to the stool examination. Additionally, I agree that part of my stool sample may be sent to a foreign laboratory for further diagnostic testing and may be reused in future research under the same project scope. With full understanding and without any coercion, I voluntarily agree to participate in this study.

Participant's Signature: _____

Name of the Person Obtaining Consent: _____

Signature of the Person Obtaining Consent: _____

Date: _____

Amharic Version

ተ.ቁ፡ _____

የካርድ ቁፅ፡ _____

ሙሉ ስም፡ _____

እኔ የተፈረመው፣ በአዲስ አበባ ከተማ አቃቂ ቃሊቲ ክልል ውስጥ በ _____ የጤና ማዕከል ላይ ስለሚካሄደው፣

“በአቃቂ ቃሊቲ የአዲስ አበባ ከተማ ውስጥ የሚገኙ ጥራዝ ከብቶች፣ ህፃናት እና የመጠጥ ውሃ ምንጮች ላይ የክሪፕቶስፖሪዲየም በሽታ ጥናት” ዝርዝሩ በበለጠ ተቀምጧል እንደሆነ አረጋግጣለሁ።

የጥናቱን ዓላማ፣ ጥቅሞች፣ አደጋዎች እና የግል መረጃ ምስጢራዊነትን በግልፅ መልኩ ተረድቻለሁ። ጥያቄ ለመጠየቅ እንደተፈቀደልኝ አውቃለሁ፣ በማንኛውም ጊዜ ከጥናቱ መውጣት መች እንዳለኝ ተረዳሁ። የተሰበሰበው የግል መረጃ በስም ሳይሆን በቁጥር መክደን በመስራት ማንኛውም ሰው ውጤቱን እንዳያውቅ ተደርጓል።

ከዚህ ውጪ ለእኔ በቀጥታ የሚደርስ ግል ጥቅም አይኖረኝም፣ ነገር ግን የፍጹም የሆነ የቁምፊ ክርክር አገልግሎት ተሰጥቶኛል። በተጨማሪም፣ የተሰበሰበው የወገብ ነጠላ አካል አንድ ክፍል ለተጨማሪ ምርመራ ወደ ውጭ አገር እንዲልኩትና ለአገናኝ የሚመጡ ምርመራዎች ላይ እንዲጠቀሙበት ምክንያት የለኝም።

ስለዚህ፣ የጥናቱን አስፈላጊነት በተረዳሁ ሁኔታ እና ያለ ኃይል ወይም ግፍ በነፃ ፈቃድ በዚህ ጥናት ላይ መሳተፌን እረጋግጣለሁ።

የተሳታፊ ፊርማ፡ _____

የፈቃድ የተወሰነበት ባለሙያ ስም፡ _____

ፊርማ፡ _____

ቀን፡ _____

ANNEX 9: *Cryptosporidium* Oocysts Positive Sample during the Laboratory in the Study