ADDIS ABABA UNIVERSITY

COLLEGE OF VETERINARY MEDICINE AND AGRICULTURE

STATUS OF CRYPTOSPORIDIUM PARASITE IN DOGS AND CATS IN BISHOFTU, OROMIA, ETHIOPIA.

MVSc THESIS

BY

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DEPARTMENT OF MICROBIOLOGY, PARASITOLOGY AND POULTRY HEALTH

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STATUS OF CRYPTOSPORIDIUM PARASITE IN DOGS AND CATS IN BISHOFTU, OROMIA, ETHIOPIA.

A thesis submitted to the College of Veterinary Medicine and Agriculture of Addis Ababa University in Partial Fulfillment of the requirements for the Degree of Master of Veterinary Science in Veterinary Parasitology

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As member of the examining board of the final MSc open defense, we certify that we have read and evaluated the Thesis entitled "**Status of Cryptosporidium Parasite in dogs and cats in Bishoftu, Oromia, Ethiopia**"

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TABLE OF CONTENTS **PAGES**

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ABBRIVIATIONS

DALYs	Disability adjusted life years
EIA	Enzyme immuno-assay
ELISA	Enzyme linked immuno-sorbent assay
LAMP	Loop mediated isothermal amplification
PCR	Polymerase chain reaction
PCR-MAS	PCR- multiplex allele specific
RFLP	Restriction fragment length polymorphism
WOAH	World organization for animal health
MZN	Modified Ziehl Nelsen
DNA	Deoxyribo Nucleic Acid

LIST OF TABLES **PAGES**

LIST OF FIGURES

ABSTRACT

Cryptosporidiosis is a zoonotic infection caused by *Cryptosporidium* spp, a coccidian protozoan parasite which is one of the most prevalent waterborne diseases. Long term co evolution and close interaction of human being with animals serving as reservoir of this parasite made the transmission way simple for the parasite. A cross-sectional study was conducted over an eight month period in and around Bishoftu town from Oct, 2024 to Jun, 2025 with the aim of estimating the prevalence and intensity of Cryptosporidium infections and identifying potential risk factors associated with the occurrence of the parasite in Pet animals. The study employed a purposive sampling strategy and included 256 pets (192 dogs and 64 cats) that were admitted to Veterinary Teaching Hospital and other veterinary clinics in the study area. Aseptically collected fecal sample were examined using modified acid fast techniques to identify Cryptosporidium oocysts. From 256 examined fecal samples, 2.34% (6/256) were found positive, with 6 (3.1%) from dogs and 0 (0%) from Cats. The current study revealed that age had a significant impact on infection rates, with the highest occurrence found in young animal less than one year (10.3%, p=0.036), compared to other age group. Furthermore, the fecal consistency also showed statistically significant difference (p=0.019) while other considered risk factors are not significant. The intensity of cryptosporidium oocysts were counted and categorized as low because the numbers of oocysts per high magnification field were below 5. However, the burden of infection between the age group was relatively high in dogs under 1year old. The study reveals notable presence of cryptosporidium in dogs rather than cats in study area, with higher risk among young animals. In conclusion this finding underscores the need for improved hygiene and public awareness about zoonotic risk of the parasite.

Key words: *Bishoftu, Cryptosporidium , Dogs, Cats, Oocysts, Prevalence, Risk factors*

1. INTRODUCTION

Humans have maintained a close and enduring relationship with their companion animals, notably cats and dogs, over countless years, offering numerous advantages to their owners and caregivers, such as enhanced physical activity, companionship, and better mental health (Martin *et al.,* 2023). Certain pet owner practices, like sharing beds with pets, permitting pets to lick their faces and hands, not washing hands frequently enough, and feeding raw meat to dogs and cats, can facilitate the spread of various zoonotic pathogens from pets to humans, including enteric protozoans like cryptosporidium and giardia (Barbosa *et al*., 2023).

In many animal species and humans, Cryptosporidiosis is a zoonotic infection brought on by *Cryptosporidium spp* a coccidian protozoan that is a member of the Apicomplexan class (Wright and Coop, 2007). It is the main cause of waterborne disease outbreaks globally and one of the most common waterborne illnesses (Helmy *et al*., 2017). Humans, farm and companion animals, wild animals, birds, and reptiles are among the vertebrates infected by these intracellular parasites of the genus Cryptosporidium (de Oliveira *et al*., 2012).

Instances of Cryptosporidium infection in dogs have been relatively rare, with most cases occurring in puppies under 6 months old. The first report of cryptosporidiosis in dogs was documented in 1981 by Tzipori and Campbell, who found Cryptosporidium antibodies in 16 out of 20 canine serum samples (Morgan *et al*., 2000). Young animals are more prone than adults to have clinical symptoms in both dogs and cats, but in cats, these symptoms can persist intermittently for extended periods of time. It has long been believed that dogs and cats are reservoirs of human Cryptosporidium infections because they are frequent hosts of *Cryptosporidium spp* (Santin, 2013). The most common species of Cryptosporidium that infect dogs and cats are *C. parvum and C.canis, C. felis*, respectively (Koompapong *et al*., 2014).

The majority of the symptomatic cases of cryptosporidiosis have been reported in animals with immune suppression, or in those that had coinfection with other infectious organisms or had preexisting diseases of the intestines like inflammatory bowel disease, lymphoma, or infections such as, canine distemper virus, or feline leukemia virus. Common clinical signs of disease in cats and dogs are small bowel

diarrhea, anorexia, and weight loss. Diarrhea is usually watery, without mucous, blood, melena, or straining (Scorza and Tangtrongsup, 2010).

Parasite transmission occurs through ingestion of oocysts, through either direct contact or consumption of contaminated water or food. Oocysts are meiotic spores and the product of parasite sex. *Cryptosporidium* has a single-host life cycle in which both asexual and sexual processes occur in the intestine of infected hosts (Tandel *et al*., 2019). Observing the infectious stage of oocysts, which are typically 4–6 µm in size, is typically necessary for the diagnosis of the infection (Connelly *et al*., 2008). A number of techniques can be used to diagnose this parasite including microscopic examination either by the wet mount preparation or staining the smears with modified acid-fast stain or by fluorescent stains. Immunological methods detecting both antigen and antibody are available. Histological examination of the biopsy and various molecular methods for detection of DNA are also available (Khurana and Chaudhary, 2018).

Because there is currently no effective treatment available, the focus for preventing cryptosporidiosis primarily involves eliminating or reducing the environmental contamination caused by infectious oocysts (Helmy and Hafez, 2022). Based on the outcomes of various medications that have been evaluated, paromomycin seems to be the preferred option for treating cryptosporidiosis in both dogs and cats according to (Shahiduzzaman and Daugschies, 2012).

Waterborne contamination is an increasing issue that leads to widespread disease outbreaks and is also recognized as a significant food-borne pathogen, resulting in diseases of considerable socioeconomic importance globally (Putignani and Menichella, 2010). Furthermore, the economic consequences of cryptosporidiosis, arising from productivity losses, are substantial. In livestock, Cryptosporidium infections can have significant economic repercussions for farmers due to high rates of morbidity and, in some cases, mortality in farm animals, particularly among the young. This can lead to serious illness or death, resulting in decreased performance and production losses, ultimately causing financial strain on producers from both the additional care and supportive treatments required, as well as the loss of production animals (Xiao *et al.*, 2004).

Despite the growing public health concern of zoonotic parasite in companion animals, there is limited information regarding the prevalence and intensity of cryptosporidium infection in dogs and cats in Ethiopia, particularly in Bishoftu. While *Cryptosporidium* species are recognized as significant enteric pathogens capable of causing diarrhea in animals and humans, local data on the occurrence, transmission dynamics and zoonotic potentials remain scarce. The lack of surveillance and baseline epidemiological data in this region hampers the ability to implement effective control strategies and assess the potential public health risks associated with pet ownership.

1.1 . Objectives

1.1.1. General Objective

- To estimate the prevalence and intensity of Cryptosporidium infections and identify potential risk factors associated with the occurrence of the parasite in dogs and cats in Bishoftu Town.

1.1.2 .Specific Objective

- To estimate the prevalence of Cryptosporidium infection in different species of dogs and cats in Bishoftu Town.
- To estimate the intensity of infection in infected dogs and cats
- To identify risk factors associated with infection in dogs and cats

2. LITERATURE REVIEW

2.1. Taxonomy and Classification of Cryptosporidium

The taxonomic status of the genus Cryptosporidium remains unclear, and its speciation presents ongoing challenges for taxonomists (Mohammed *et al.,* 2017). Cryptosporidium is classified within the phylum Apicomplexa, class Sporozoasida, subclass Coccidiasina, order Eucooccidiida, suborder Eimeriina, and family Cryptosporidiidae (Bamaiyi and Redhuan, 2016). To date, at least 22 species of Cryptosporidium have been identified based on factors such as host occurrence, parasite morphology, host preference, and site of infection (Ramirez *et al*., 2004).

2.2. Etiology

The ingestion of environmentally resistant oocysts is the etiology of Cryptosporidium infections in dogs, cats. These oocysts can be spread through contaminated surfaces, food, water, direct contact with infected animals, or their excrement (Santín *et al*., 2011). *Cryptosporidium canis* and *Cryptosporidium felis* are the most commonly found species causing infections in dogs and cats, respectively. Due to their high resistance to environmental factors and disinfectants, the oocysts are widespread and challenging to control. Once ingested, the oocysts transform into sporozoites, which invade the epithelial cells of the gastrointestinal tract, leading to clinical symptoms such as diarrhea, dehydration, and weight loss, especially in young, stressed, or immunocompromised animals (Thompson *et al*., 2008).

2.3. Epidemiology of Cryptosporidium

2.3.1. Distribution of Cryptosporidium in Dogs

The distribution of Cryptosporidium infection in dogs is a subject of considerable interest due to its zoonotic potential and the widespread presence of the parasite. Several studies have shown that Cryptosporidium can infect dogs globally, with varying prevalence rates depending on geographic location, age, and living conditions of the dogs (Oner and Ulutaş, 2022). Research conducted in European countries such as Germany and the United Kingdom reported prevalence rates ranging from 1% to 10% in both pet and shelter dogs (Lucio-Forster *et al*., 2010).

Environmental influences are crucial in determining the prevalence of Cryptosporidium infections in dogs. Dogs situated in rural or semi-rural regions, particularly those with access to polluted water supplies, face an increased likelihood of infection. Dogs in urban settings although less frequently exposed to natural water sources, can still contract the parasite through contact with fecally contaminated surfaces or ingestion of contaminated food and water (Thompson *et al*., 2008).

The age of a dog and the state of its immune system significantly influence the spread and severity of Cryptosporidium infections. Young puppies are at a higher risk of infection and often display more severe symptoms due to their underdeveloped immune systems. In contrast, adult dogs, with their fully developed immune systems, may carry the parasite without showing any symptoms, serving as silent carriers that facilitate transmission (Gow & Waldner, 2006). According to Taghipour, *et al. (*2020), the prevalence of Cryptosporidium reported using molecular detection methods was highest in pet (domestic) dogs 9% followed by mixed dogs 6% and stray (wild) dogs 6%. (Table 1 shows distribution of cryptosporidium globally).

Table 1. Prevalence of cryptosporidium in dogs in different country

Country	**Methods**	**Prevalence (%)**	**Reference**
Nigeria	MZN	22.75	Chukwu *et al*., 2019
Nigeria	MZN	36.5	Eze *et al.*, 2019
Zambia	MZN	5.9	Mugala *et al.*, 2018
Iran	MZN	3.7	Jokar *et al*., 2021
Japan	PCR	9.3	Abe *et al*., 2002
Germany	PCR	10	Murnik *et al*., 2022
America	PCR	21	Naoyuki *et al*., 2019
Globally	Meta-analysis	9	Taghipour *et al*., 2020

2.3.2. Distribution of Cryptosporidium in Cats

The presence of Cryptosporidium in cats differs across the globe, shaped by factors like geographic area, living environments, and the age of the cats as shown in table 2. *Cryptosporidium felis* is the main species affecting cats, with prevalence rates

reported between 1% and 6% across various regions. Certain studies have indicated higher rates of infection in cats residing in multi-cat households, shelters, or settings with inadequate sanitation (Lucio-Forster *et al*., 2010). Young kittens and immune compromised cats are particularly susceptible to infection, often exhibiting symptoms such as diarrhea, which can lead to dehydration and weight loss if untreated. In contrast, adult cats with robust immune systems may carry the parasite asymptomatically, contributing to the spread of oocysts in the environment (Thompson *et al*., 2008).

Table 2: Prevalence of Cryptosporidium infection in cats in different country

Country	**Methods**	**Prevalence (%)**	**Reference**
Mexico	MZN	7	Iturbe *et al*.,2021
Iran	Meta-analysis	8.8	Joker *et al*., 2021
South Africa	PCR	32	samie *et al*., 2013
South America	Meta-analysis	4	Taghipour *et al*., 2021
Central America	Meta-analysis	4	Taghipour *et al*., 2021
Africa	Meta-analysis	14	Taghipour *et al*., 2021
Globally	Meta-analysis	6	Taghipour *et al*., 2021

2.3.4. Risk Factors

Intrinsic factors: It is protected by an outer shell that allows it to survive outside the body for long periods of time and makes it very resistant to chlorine disinfectants (Kosek *et al*., 2001). As a result, the oocysts of the Cryptosporidium can survive for several months and retain infectivity in a latent form outside the host, despite adverse environmental factors, including salinity and chemicals (Sunnotel *et al*., 2006; Smith *et al*., 2007). Other intrinsic factors include its ability to undergo both asexual and sexual reproduction within a single host and facilitating rapid propagation and transmission. Additionally, *Cryptosporidium* exhibits a high degree of genetic variability, aiding in its adaptability and resistance to host immune responses and potential treatments and *Cryptosporidium* oocysts are immediately infectious because they do not need to sporulate outside the host (Ryan *et al*., 2016; Zuo *et al*., 2023)..

Important predictors of the results of host-pathogen interactions are host factors. The severity of a Cryptosporidium infection has been shown to be strongly influenced by host characteristics, including age, immunological function, genetic susceptibility, and general health (Petry *et al*., 2010). Age is a significant determinant of susceptibility to *Cryptosporidium* infection, Studies have shown that higher infection rates and more severe clinical symptoms in younger animals compared to adults (Berhanu *et al.,* 2022).

Neonates and young animals are particularly vulnerable due to their underdeveloped immune systems, which are less capable of effectively combating the parasite. For instance, puppies, kittens, and young birds exhibit a greater prevalence of *Cryptosporidium* infections, often resulting in severe diarrhea and dehydration (Ryan, 2010). Other characteristics that might be considered include immune state and general health. The detection of Cryptosporidium in two Australian domestic dogs under immunological stress was hypothesized to be the result of canine distemper, which suppressed the immune system and made the dogs more vulnerable to cryptosporidial infections (Morgan *et al*., 2000).

Extrinsic factors: Management practice inadequate hygiene, overcrowded living conditions, and poor sanitation are significant risk factors that facilitate the transmission of *Cryptosporidium* (Putignani and Menichella, 2010). For pets like dogs and cats, regular cleaning and disinfecting of living areas, food, and water bowls are essential preventive measures of cryptosporidiosis (Mugala, 2016). In addition to management practice and other extrinsic factors, a seasonal incidence of infection is sometimes present, possibly corresponding to rainfall peaks, increased pollution from farm waste, or calving and lambing activities (Wilkes *et al*., 2009).

2.4. Life Cycle and Transmission

Cryptosporidium spp. has monoxenous life cycles, where all stages of development (asexual and sexual) occur within one host (Smith *et al*., 2007). All species of Cryptosporidium undergo endogenous development, resulting in the production of an encysted stage discharged in the feces of their host (Fayer *et al*, 2000). The sporozoites enter the microvilli of various epithelial cells, whereas the oocysts expel themselves in the digestive system. Cryptosporidium species determine where the

infected host cells are located. The elongated sporozoite turns into a trophozoite by rounding up. Eight type I merozoites are produced by the multinucleated type I schizont trophozoite, which then pass through further microvilli to create type II schizonts. Type II schizonts repeat this developmental cycle, resulting in four type II merozoites. Recycling of asexual stages may occur. Type II merozoites penetrate microvilli and become sexual stages.

Nonflagellated microgametes are produced by the male stage, also known as the microgamont. Oocysts are created during fertilization in the female stage, also known as the macrogamont. There are two kinds of oocysts that are formed; they both sporulate endogenously and have no sporocysts, four sporozoites, and an oocyst residuum. Oocysts with thin walls can spread by themselves. The feces contain thick-walled oocysts. Consumption of oocysts, which contain four sporozoites each, starts the infection. These sporozoites hatch at the intestinal level and release infectious sporozoites (Tzipori and Widmer, 2008).

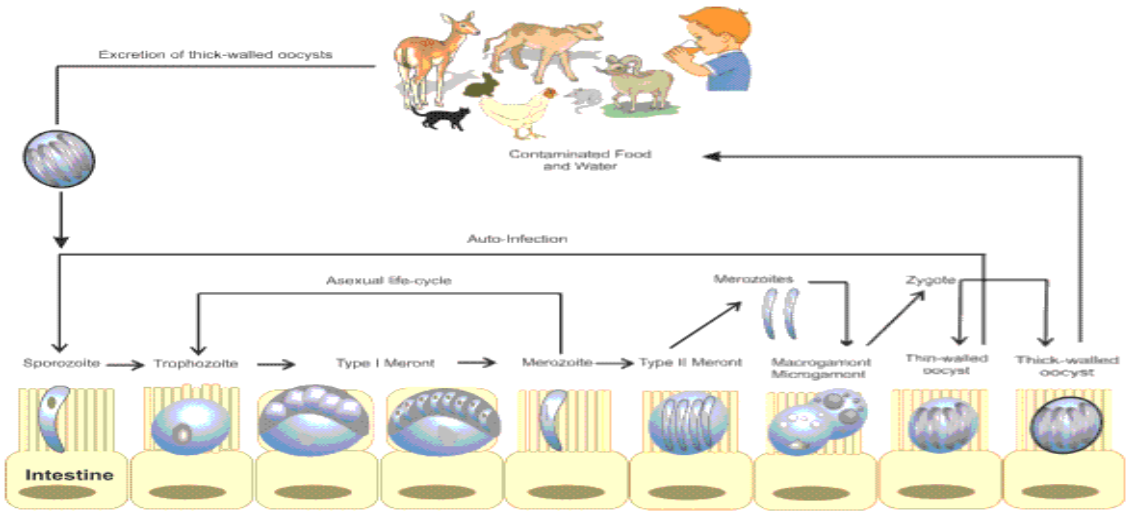

Figure 1: Life cycle of Cryptosporidium in different animal species (Yosra, 2014).

2.5. Pathogenesis and Clinical Sign

Once the thick-walled oocyst is consumed through food or water by the host, various signaling molecules are produced on the surface of the sporozoite that facilitate its attachment and penetration into host cells. It has been observed that calcium-dependent protein kinases (CDPKs) play a role in regulating the sporozoite's invasion

of the host cell. Additionally, instead of directly invading the host cells, Cryptosporidium is internalized by the host cell (Helmy and Hafez, 2022). As a result, it remains in an epicellular position, leading to significant actin reorganization in the infected cells following the attachment and invasion of Cryptosporidium; the interactions between the host and parasite are crucial in the development of the disease (Tzipori and Ward, 2002).

Cryptosporidium spp lead to atrophy and fusion of enterocyte microvilli, accompanied by local inflammation. This reduces the surface area available for absorption, causing apoptosis of enterocytes in the host. The resulting pathogenesis creates an imbalance in nutrient transport (da Silveira-Neto *et al*., 2015). In immunocompetent individuals, the disease is usually a self-limiting acute gastroenteritis, characterized by vomiting, weight loss, fever, watery diarrhea, cramping, abdominal pains, flatulence, malaise and myalgia (Chin, 2000).

2.6. Diagnosis

The optimal diagnostic method for isolating and identifying Cryptosporidium varies based on the intended purpose and the diagnostic capabilities at hand (Ahmed and Karanis, 2018). For instance, a wet mount combined with specific stains—such as acid-fast dye, fluorescence, or immunofluorescence can effectively detect oocysts in stool samples (Sarkar *et al*., 2014). Several factors influence the choice of diagnostic technique, including the level of technical expertise, the necessary sensitivity and specificity, time constraints, and budget limitations (Chalmers and Katzer, 2013).

A variety of tests have been developed for the diagnosis of Cryptosporidium most of them involve direct detection by microscopic examination of tissues or fecal material using staining techniques (Ramirez *et al*., 2004). The diagnosis of cryptosporidiosis in a laboratory setting is essential for the effective management of patients, whereas in environmental samples, it is typically necessary for pinpointing outbreaks, tracking sources, evaluating risk factors, and implementing interventions (Khurana and Chaudhary, 2018).

2.6.1. Microscopical diagnosis

Wet mounting and staining techniques: Both the preserved and unpreserved stool samples exhibit Cryptosporidia. Polyvinyl alcohol (PVA), sodium acetate formalin, or 10% formalin should be used to preserve the samples that are thought to be delayed. Under light microscopy using wet mounting methods, they appear as smooth, colorless, spherical or slightly oval entities that range in size from 3 to 8 μm (Khurana and Chaudhary, 2018).

Romanowsky stains, including Giemsa and Jenner's stain, were among the first utilized for identifying oocysts. The oocyst appears semi-translucent, featuring a narrow clear halo around it, and exhibits blue to azure coloring with four to six red or purple eosinophilic granules that look like dots. (Petry. 2000). Due to the tiny size of these oocysts, differential staining using the modified Ziehl-Neelsen technique and wet mount preparation methods have limited value for the detection of Cryptosporidium in faecal samples where oocysts can easily be confused with other materials present in the samples (Connelly *et al*., 2008).

2.6.2. Immunological techniques

Although immunological tests are more expensive, they utilize monoclonal antibodies developed for specific targets, allowing for species differentiation and offering exceptional sensitivity compared to conventional staining methods. Immunological diagnosis relies on either the detection of antigens or antibodies. Detecting antigens indicates an active infection, while detecting antibodies may suggest a past infection; however, this approach is valuable for seroepidemiological studies (Khurana and Chaudhary, 2018). Enzyme immunoassay (EIA) has been effectively used to detect antigens in fecal samples for Cryptosporidium screening (Garcia *et al*., 2000). The application of ELISA to identify cryptosporidial antigens in stool specimens is advantageous for screening large volumes of samples (Agnamey *et al*., 2011).

2.6.3. Molecular techniques

For many years, conventional procedures were the primary diagnostic techniques for cryptosporidiosis in most laboratories worldwide, followed by immunological

approaches. These methods had limitations in sensitivity and specificity due to being labor-intensive, time-consuming, requiring skilled technicians, and being susceptible to false-positive and false-negative results. The introduction of PCR, which offers greater sensitivity and can detect as few as 1 to 10^6 oocysts, has transformed diagnostic laboratories (Smith, 2007). Several nucleic acid detection techniques are now in use, including PCR-restriction fragment length polymorphism (PCR-RFLP), multiplex allele-specific PCR (MAS-PCR), and quantitative real-time PCR. Additionally, loop-mediated isothermal amplification (LAMP) has emerged as a valuable diagnostic method for various organisms, including Cryptosporidium species, due to its simplicity and specificity (Smith, 2007).

2.7. Treatment

As it stands today, available treatments for the treatment of cryptosporidiosis in dogs and cats are limited, and there are no universally accepted, effective drugs for eradicating the infection. For over two decades there has been a credible effort to investigate chemotherapeutic options for Cryptosporidium infections in both animals and humans. More than 100 agents have been investigated in laboratory animal models, but none have proven to be significantly effective clinically. Supportive care is the main treatment, aimed at symptom management and control of dehydration. Fluid therapy may be indicated for adequate hydration and electrolyte balance in severely dehydrated animals that have pronounced diarrhea (Santín *et al*., 2011).

One specific medication, paromomycin, has shown some efficacy in reducing oocyst shedding and alleviating clinical symptoms in dogs and cats, although its use is not universally approved or available (Fayer *et al*., 2001). Other drugs, such as Nitazoxanide and azithromycin, have been tested with varying success. Nitazoxanide, an antiparasitic drug, has shown promising result in treating cryptosporidiosis in humans and has been used off-label in pets, but it can have significant side effects and is not always effective (Panciera *et al.*, 2000).

2.8. Prevention and Control

Understanding the distribution of Cryptosporidium species and subtypes, mechanisms of transmission, and sources of infection is crucial for disease prevention and control (Pal *et al*., 2021). One of the most effective preventive measures is keeping pets'

living spaces clean, which includes routinely cleaning and disinfecting food and water bowls, litter boxes, and bird cages with potent disinfectants. Preventing and controlling Cryptosporidium infection in dogs and cats requires a multifaceted approach that includes environmental management, good hygiene practices, and routine veterinary care (Santín *et al.,* 2011). *Cryptosporidium* oocysts are highly resistant to many common cleaning agents (Lasprilla-Mantilla *et al*., 2019).

2.9. Public Health and Economic Importance of Cryptosporidium

Cryptosporidiosis, the disease caused by *Cryptosporidium*, is a leading cause of waterborne outbreaks globally, often associated with contaminated drinking water and recreational water sources (Fayer, 2010). Cryptosporidiosis has emerged as a significant public health issue in the developed world. For example, the Disability-Adjusted Life Years (DALYs) estimate suggests that the disease burden of Cryptosporidium in the Netherlands, despite proper sanitary practices, has increased since the last assessment (Golomazou *et al*., 2024).

In developing country the situation is further aggravated by a lack of access to clean water and sanitation, leading to high mortality and morbidity, especially in children under five years of age (Kotloff *et al.,* 2013). Study have shown that epidemiological surveys have shown that the prevalence of cryptosporidiosis in developing nations (5 % to >10 %) is higher than that in developed nations (<1–3 %), primarily due to poor water quality and food sanitation (Karanis, 2018).

The economic impact of cryptosporidiosis on producers arises from its high morbidity and, at times, mortality in farm animals. In young animals, cryptosporidiosis can lead to severe disease or death, resulting in decreased performance and loss of production. This situation causes economic losses for producers due to the increased care, supportive therapy needed, and the mortality of production animals (Xiao *et al*., 2004; WOAH, 2022).

2.10. Status of Cryptosporidiosis at Global and National Level

The diagnostic procedures employed can result in differences in the sensitivity and specificity of the results, which in turn can cause differences in the prevalence rates of cryptosporidiosis around the world. Several studies have investigated the prevalence

of *Cryptosporidium* and have reported a wide range of estimates in different settings (Ahmadpour *et al.,* 2020). Study conducted by Taghipour *et al*. (2020) has shown that the overall prevalence of Cryptosporidium infection was estimated at 8% (95 % CI: 5 -11%) using microscopic methods, 7% (95 % CI: 4-10%) using coprogantigenic methods and 6% (95 % CI: 4-9%) using molecular diagnostic methods globally. In cats, the pooled global prevalence (95% CI) of Cryptosporidium spp. was 6% (4–8%), with the highest prevalence occurring in Africa (14% (0–91%) and the lowest in South and Central America (4%–7%), according to Taghipour *et al*. (2021). in Ethiopia, to the best of the author knowledge, there is no published report regarding cryptosporidium prevalence in small animals.

3. MATERIAL AND METHODS

3.1. Description of Study Area

Bishoftu town is located at 9°N latitude and 40°E longitudes at an altitude of 1850 m above sea level in central mid lands of Ethiopia. It has an annual rainfall of 866 mm of which 84% is in the long rainy season (June to September). The dry season extends from October to February. The mean annual maximum and minimum temperatures are 26 °C and 14 °C respectively, with mean relative humidity of 61.3 %(NMA, 2007). According to Ayehu. (2022) The total dog population in Bishoftu town is estimated to be 4188 (Bayou *et al*., 2025) there is no specific report on the population of cats in the study area yet .

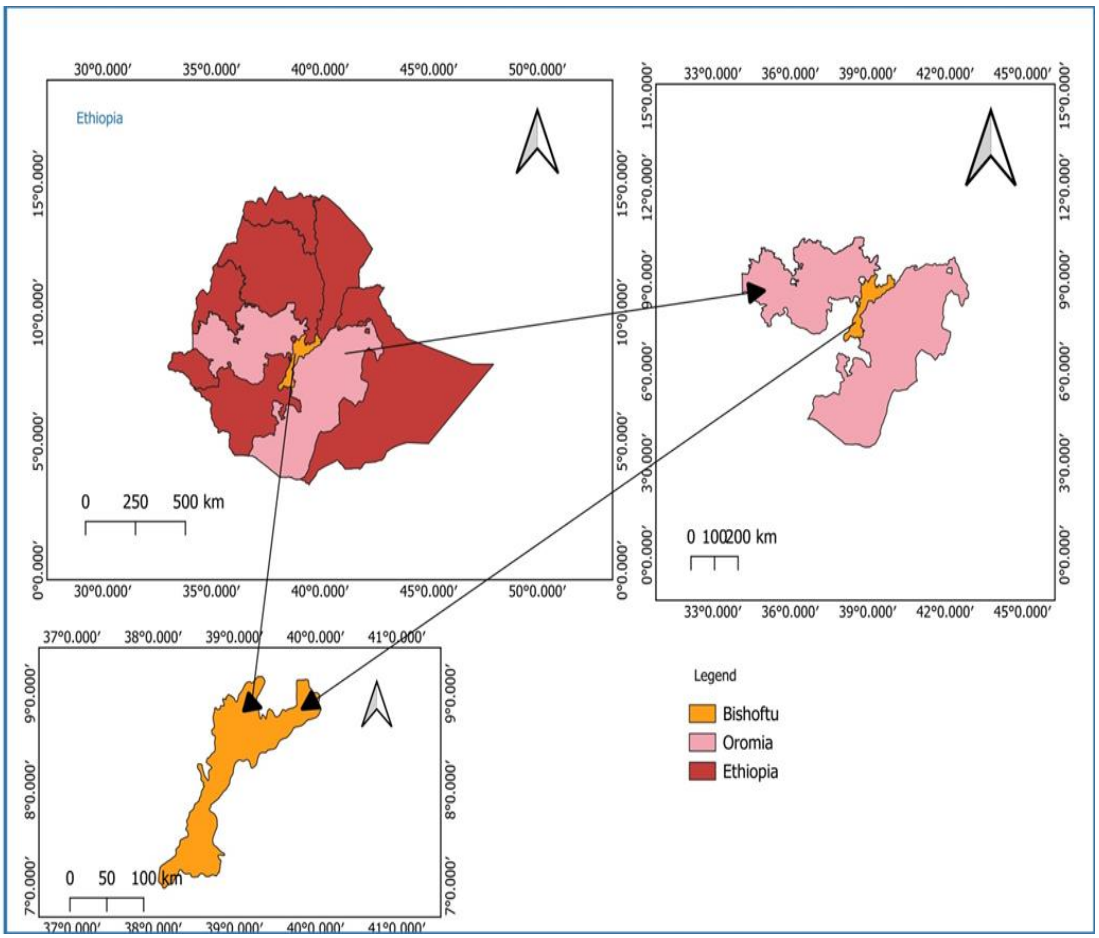

Figure 2: Map of the study areas prepared using QGIS 3.40.6

3.2. Study Population

The study focuses on pet dogs and cats residing in Bishoftu Town, Oromia, and Central Ethiopia. These animals were selected as they are commonly kept as pets in the region and have been identified as potential reservoirs for Cryptosporidium. The study was conducted on dogs and cats of different age and sex kept under different management systems and collected from dogs and cats admitted veterinary teaching hospital and veterinary clinics in the vicinity area. The study populations included dogs of both sexes and all age groups that were kept under different management systems from the vicinity of investigations.

3.3. Study Design and Sampling

The cross-sectional study was conducted in Bishoftu town from October, 2024 to June, 2025. Fresh fecal samples were collected purposively from entire dogs and cats brought to veterinary teaching hospital of the college, and veterinary clinics during the study time accordingly, the sample were collected from 192 dogs and 64 cats totally 256 pet animal were sampled for the study. A structured questionnaire was utilized to gather demographic details about the dogs and cats, as well as management factors including feeding practices, whether the dog was confined, the number of dogs in the household, and sources of water, among other aspects

3.4. Sample Collection

Using sterile disposable gloves, the sample was taken directly from the rectum. Before being transported in an ice box to the parasitology laboratory at Addis Ababa University, College of Veterinary Medicine and Agriculture for analysis, the samples were placed in labeled universal bottles and preserved in 10% formalin at a ratio of 1 gram of feces to 3 ml of formalin to prevent the desiccation of Cryptosporidium oocysts. Conversely, dogs that live in kennels or on chains but have access to unfenced compounds and can roam the neighborhood after being released are categorized as semi-intensive. Dogs that live in kennels within a fenced compound and are permitted to move only within the compound upon release are classified as having intensive management practices.

3.5. Laboratory Analysis

3.5.1. Screening for Cryptosporidium

Flotation techniques using concentrated sugar solution of specific gravity 1.26 and modified acid fast staining were used to detect the oocysts of Cryptosporidium. Fecal flotation and acid-fast staining are essential laboratory techniques used for the diagnosis and screening of Cryptosporidium oocysts (Rekha *et al*., 2016). The fecal flotation method aims to concentrate oocysts from fecal samples, allowing for the differentiation of Cryptosporidium from other parasites. In this process, a fecal sample is mixed with a flotation solution that has a higher specific gravity than the oocysts. On the other hand, acid-fast staining is specifically designed to visualize Cryptosporidium oocysts, which possess a thick, acid-fast wall. This technique involves preparing a fecal smear on a microscope slide, fixing it, and applying an acid-fast stain such as the Ziehl-Neelsen stain. After rinsing, the slide is treated with an acid-alcohol solution, allowing the oocysts to retain the stain and appear red against a blue or green background when viewed under a microscope (Garcia, 1999).

3.5.2. Assessment of Oocyst Intensity.

Cryptosporidium oocyst intensity was estimated using the average number of oocysts (oocyst per field) in 10 randomly chosen fields at 100X magnification. Oocyst intensity was categorized as low (1–5 oocysts), medium (6–10 oocysts), and high (more than 10 oocysts) (Ayana *et al.*, 2022).

3.6. Data Management and Statistical Analysis

All data collected from study animals and laboratory results was entered and stored in a Microsoft Excel spreadsheet program. The statistical analysis performed using stata software14. Chi-square test and Mann-Whitney Test of non-parametric values tests were utilized to analyze the data. Logistic regression analysis also used to identify the potential risk factors associated with the occurrence of Cryptosporidium infection at a desired precision level of 5% and confidence interval of 95%. Significant difference was considered when $P \leq 0.05$.

3.7. Ethical Clearance

This study was granted ethical approval by the College of Veterinary Medicine Animal Research Ethics Committee of Addis Ababa University, with reference number **VM/ERC/04/81/17/2025** (ANNEX 4). Every procedure was carried out by qualified experts in compliance with the rules and norms set forth by the university's ethical committee. The animals who took part The current study were kept safe and healthy during the entire investigation. All pet owners gave their verbal agreement before the study started in order to collect fecal samples from their animals.

3.8. Limitation of the Study

Current study has its own limitation among those: difficulties in managing and collecting samples from the study population especially from cats are the crucial one. Additionally, microscopic techniques like Modified Ziehl-Neelsen (MZN) have limited sensitivity and specificity. PCR, while more accurate, was not applied to all samples due to cost and availability constraints.

4. RESULTS

The overall prevalence of Cryptosporidium infection was 2.34% (6/256) in pets, specifically 3.1% in dogs while no cases were detected in cats (0/64). Age had a significant impact on infection rates, with the highest occurrence found in young animals less than one year (10.3%, p=0.036), compared to lower rates in those aged 1–3 years (1.61%, p=0.038) and over three years (0.97%, p=0.036). Sex didn't show a statistically significant difference, with infection rates of 2.3% in males and 2.36% in females (p=0.980). Feeding practice and management system methods were linked to infection risk: pets consuming raw food had a higher prevalence (4%, p=0.118), as did those managed under semi-intensive systems (4.2%, p=0.105). Pets showing signs of diarrhea were more frequently infected (6.8%, p=0.019) than those without diarrhea (0.54 %,). Furthermore, pets that had contact with other animals faced an increased risk of infection (4%, p=0.11) (**Table 3)**.

Table 3: Over all prevalence of cryptosporidium in in the study.

Variable	**Category**	**No of examined**	**Number of positive**	**Prevalene (%)**	**P value**
Species	Dogs	192	6	3.1	-
	Cats	64	0	0	
Age	<1 year	29	3	10.3	0. 036*
	1-3 year	124	2	1.61	
	>3year	103	1	0.97	
Sex	Male	129	3	2.3	0.980
	Female	127	3	2.36	
Feed	Cooked	133	1	0.75	
	Uncooked	123	5	4	0.118
Managemen t	Intensive	137	1	0.72	
	Semi-intensive	119	5	4.2	0.105
Fecal consistency	Diarrheic	73	5	6.8	
	Non-diarrheic	183	1	0.54	0.019*
Contact	Yes	123	5	4%	0.11
	No	133	1	0.75	

*Significant difference

Univariate logistic regression analysis revealed several factors that were significantly linked to Cryptosporidium infection in companion animals. As shown in **Table 4**, age had significant association with cryptosporidium infection rate, with the highest likelihood of infection found in pets younger than one year. When compared to this age group, the odds of infection were notably lower in those aged 1 to 3 years (OR = 0.14, 95% CI = 0.022–0.859, p = 0.038) and in those older than 3 years (OR = 0.08, 95% CI = 0.008–0.850, p = 0.036). Dogs were significantly more likely to be infected than cats (OR = 0.03, 95% CI = 0.008–0.076, p < 0.001). However, sex did not appear to influence infection risk (p = 0.985). Additionally, animals without diarrhea had significantly lower odds of infection (OR = 0.074, 95% CI = 0.009–0.651, p = 0.019).

Table 4: Univariate logistic regression analysis on cryptosporidium and risk factor in dogs and cats.

Variables	Category	No examined	No positive	OR	95 %CI	P-value
Age	<1years	29	3	11.76	0.0248–1.107	0.036
	1-3 years	124	2	0.14	0.0223-0.859	0.038*
	>3years	103	1	Ref		*
Sex	Male	129	3	0.98	0.1948-4.969	0.985
	Female	127	3	Ref		
Species	Dog	192	6	0.03	0.0076-0.076	0.000*
	Cat	64	0	Ref		
Management	Intensive	137	1	Ref		
	Semi Intensive	119	5	5.9	0.686-51.7	0.105
Feed	Cooked	133	1	Ref		
	Uncooked	123	5	5.5	0.644-48.5	0.119
Fecal Consistency	Diarrheic	73	5	13.38	1.5-116	0.019*
	Non-Diarrheic	183	1	0.074	0.0085-0.651	
Contact with other animals	Yes	123	5	5	0.644-48.7	0.118
	No	133	1	Ref		

*Significant difference, OR= odd ratio, CI= Confidence interval.

The multivariate logistic regression analysis illustrated two main factors significantly associated with Cryptosporidium infection. Dogs under <1years has higher odds of infection compared to those above >3 old, this difference approached reach statistical significance (OR = 11.76, 95% CI = 0.0248–1.107, p = 0.064). Likewise, pets older than 1-3 years showed a trend toward reduced infection risk, with results not statistical significance (OR = 0.1, 95% CI = 0.15-18.7, p = 0.67). Among the variables assessed, fecal consistency was a significant independent predictor, with non-diarrheic animals significantly less likely to be infected (OR = 13.8, 95% CI = 1.692–116, p = 0.019) (**Table 5**)

Table 5: Multivariate logistic regression analysis of the assumed risk factors for cryptosporidium infection

Variables	**Categories**	**Odd ratio**	**95% confidence interval**	**P value**
Age	< 1year	11.76	1.17-117	0.036*
	1-3 year	1.7	0.15-18.7	0.67
	>3 year	Ref		
Fecal Consistency	Diarrheic	13.38	1.69-116	0.019*
	Non-diarrheic	Ref		

Infection intensity/burden of Cryptosporidium parasite

The oocyst of cryptosporidium were detected and counted under microscope. Accordingly, the samples from dogs were analyzed based on different group to evaluate the association between oocyst intensity with respective variables. The oocyst intensity recorded as low because the entire counted samples were found with less than five oocysts. The association between age group and fecal consistency with oocyst intensity show significant difference. While the other variables (sex, fed, contact with other animal and management practice) statistically not significant difference.

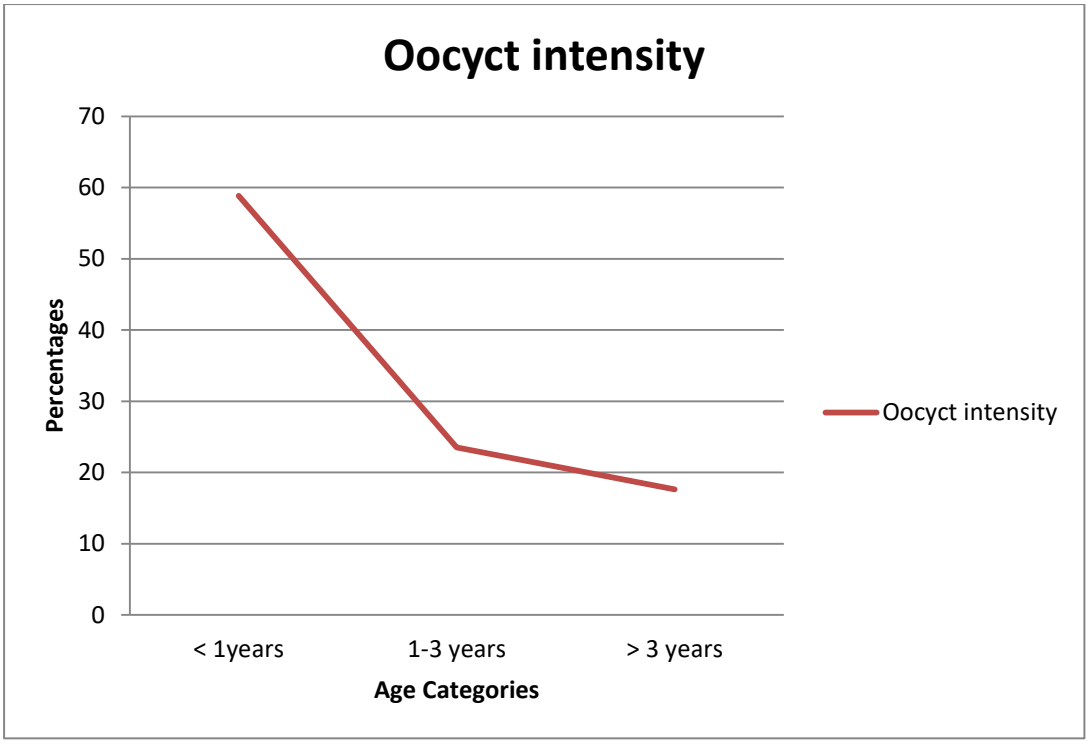

Figure 3: Age wise intensity of cryptosporidium

4. DISCUSSION

According to the studies conducted in different countries worldwide, the estimate prevalence of canine *Cryptosporidium* oocysts vary from 1% to 20% and some factors, such as geographic location, status of animal ownership, sampling protocols, demographic factors, anthelmintic use and diagnostic techniques are responsible for the wide range of *Cryptosporidium* prevalence (Mirzaei., 2012). In current study the overall prevalence of cryptosporidium in pet animals was determined in Bishoftu town, to be (2.34%) this result closely in line with reported result (3.91%) in Pakistan by (Khan *et al*., 2023). However this finding varies from the prevalence reported in South Africa (38%) by (samie *et al*., 2013).

This significant variatiation could be due to different diagnostic method used. Specifically the individual prevalence at species level revealed by this study is 3.1% in Dogs (6/192) which is higher and comparable with that detected microscopically and reported (2.7%) in Poland by (Piekara-Stępińska *et al.* (2021), however higher prevalence have been reported by different scholars at different time and place. 5.9%, 22.5% by (Mugala *et al.* (2016), Chukwu *et al.*, 2019) respectively. Even though the cats population had included in the study the finding shows 0 % (0/64) prevalence which almost in line with the result(1%) reported by Tzannes *et al*. (2008), at united kingdom.

The prevalence of *Cryptosporidium* spp. was higher in dogs under the age of 1 year compared to lower rates in those aged 1–3 years (1.61%, p=0.038) and over three years (0.97%, p=0.036). which coincides with what (Ramirez *et al*., 2004, Tangtrongsup *et al*., 2017; Khan *et al*., 2023). Despite that several study agrees on the highest prevalence in younger age than that of the old one the study reported by Oner *et al*. (2022) shows dogs of older age with higher prevalence when compared to those under 1 year of age.

The infection prevalence of *Cryptosporidium* spp. in male and female dogs The current study was 2.3% and 2.36%, respectively. Comparatively our finding is much higher than that reported (1.93% and 2.1%), respectively in Iran by Mirzaei *et al*., (2012). The sex of the dog had no influence on Cryptosporidium infection, as reported

by Jian *et al*. (2014) although the prevalence was relatively higher in female dogs than in male dogs in the current study the same relatively higher prevalence was reported by Mugala *et al* (2016) (6.8%), (5.3%) in female and male respectively. In contrary to our finding according to reported prevalence by Chukwu *et al.* (2019) and Oner *et al*. (2022) Prevalence in male (27%) relatively higher than that of female (17%) which is statistically significant Reduced immunity at certain periods in females' physiologic cycle could be taken as a reason (Olabanji *et al.,* 2016).

Feeding practice and management system methods were linked to infection risk: pets consuming raw food had a higher prevalence (4%, p=0.000), as did those managed under semi-intensive systems (4.2%, p=0.000) this result comparably agrees with Bayou *et al*. (2025).

Faeces were categorized by the consistency as diarrhoeic (73/256) and non- diarrhoeic (183/256). Diarrhoea was recorded in five of the positive samples. Prevalence of cryptosporidia was greater in diarrhoeic (6.8%) than in non- diarrhoeic (0.54%). Dogs with diarrhea were shown to have a higher chance of developing cryptosporidiosis than dogs without diarrhea. However the study reported by Moreira *et al*. (2018); Mugala *et al.* (2018) differ from our finding cryptosporidium infection was only detected in dogs without diarrhea. In comparing interactions with other animals (dogs and cats), even if the prevalence is higher in those having contact with other the results didn't show any significant difference (p = 0.11), thus agreeing with the results from Balassiano *et al.* (2009).

Our multivariate analysis revealed interesting trends in terms of pet age and risk of infection, though these were not always statistically significant. Pets between the age of >3 years had a significant reduction in the risk of infection in them compared to pets that were younger than one year old (OR = 12), an association which fell short of statistical significance (p=0.064). The same was seen in animals over 1-3 years, who similarly showed a trend towards a reduced risk of infection (OR = 0.01), though once again this narrowly failed to reach statistical significance (p=0.054). Although these age differences were not significant according to traditional standards, the fact that both sets of odds ratios pointed in the same direction indicates that age may exert a protective effect against infection.

In contrast with the age variables, fecal consistency emerged as a statistically significant independent risk predictor of infection. Non-diarrheic animals were significantly less likely to be infected (OR = 0.08, 95% CI = 0.0092–0.738, p=0.019) compared to diarrheic stool animals. This finding indicates that integrity of the gastrointestinal tract is a factor in overall susceptibility to infection. Diarrhea alone is merely a symptom of numerous infections, but this study shows that fecal consistency without diarrhea is a sensitive indicator of reduced risk of infection, perhaps pointing to superior gut microbiome condition or absence of severe enteropathogens. This supports the observation of fecal consistency as a significant marker of pet health and perhaps an early warning sign for infection.

In the present study, microscopic examination revealed the presence of Cryptosporidium oocysts, with counts ranging from 2 to 4 oocysts per field. Given the narrow range, oocyst intensity was reported descriptively rather than categorically. Among the positive samples, the highest oocyst count (4 oocysts per high-power field, or HPF) was observed in animals less than 1 year of age, while counts of 3 oocysts/HPF and 2 oocysts/HPF were recorded in animals aged 1–3 years and those over 3 years, respectively. This finding suggests a higher burden of infection in younger animals. In several study the intensity of cryptosporidium has been evaluated and oocyst burden were categorized as low, moderate and high if the number of cryptosporidium oocyst per field counted to be 1-5, 6-10, and above 10 respectively. However in current study, narrow range oocyst makes the finding difficult to compare with previous finding.

6. CONCLUSION AND RECCOMMENDATIONS

In conclusion, this study is the first to investigate the status of *Cryptosporidium* parasite infection in dogs and cats in Bishoftu, Oromia, Ethiopia. The overall prevalence was low but notable presence of the parasite in the apparently healthy pet population. Younger animals (under one year of age) and those with diarrheic fecal consistency were significantly more likely to be infected. Additionally, animals raised under intensive management systems showed a lower prevalence, suggesting that controlled housing and better hygiene practices may reduce infection risk. The findings emphasize the potential role of domestic pets as reservoirs of *Cryptosporidium* in the area, which could have public health implications given the zoonotic nature of the parasite. Based on the above conclusion the following recommendations have been forwarded;-

- Awareness among pet owners and the general public about the zoonotic risks posed by Cryptosporidium to promote responsible pet ownership and prevent transmission to humans should be given.
- Further studies using advanced diagnostic techniques to assess the potential zoonotic risks posed by infected pets to human populations, particularly in areas with close human-animal interactions should be conducted.

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8. ANNEXES

Annex 1: Data collection check list

Animal id	Species	sex	age	Feed type	Fecal consistency	Management	Contact with other animal	Result

ANNEX II: Laboratory procedures for study

Acid fast staining

- Make a thin faecal smear and allow to air dry
- Fix with absolute methanol for 10 min and allow smear to dry
- Stain with cold Kinyoun's carbol fuchsin strong stain (filtered) for 5 min
- Wash thoroughly in tap water until no further stain comes out (very important step that can take 3-5 min)
- Decolourise in 3% HCL (for very thin smears a rapid dip in Coplin jar of acid followed by an immediate rinse in tap water is sufficient)
- Counterstain with 3% Malachite green for 2-5 min
- Wash in tap water and blot dry
- Examine under a light microscope at high power (40x, 100x) for oocysts

Fecal Floatation

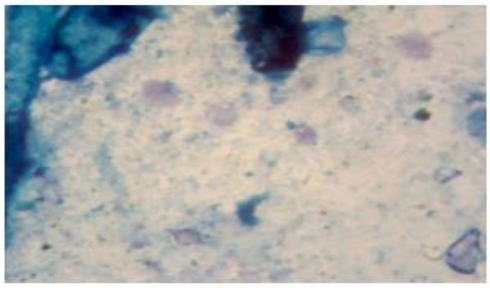
- Mix about 3 gm of feces with 42 ml of flotation solution in a plastic cup or other suitable containers.
- Strain through a tea strainer into a second cup.
- Swirl cup and decant fecal suspension into a centrifuge tube or other straight-sided vial.
- Fill tube or vial with enough flotation solution so that the meniscus is just level with the top of the tube.
- Place glass slide or coverslip on top of the tube and allow it to stand for at least 20 minutes.
- Coverslip, invert, cover drop with a coverslip and examine under 40x and 100X magnification,

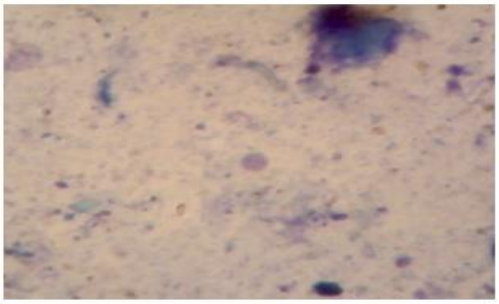
ANNEX III picture during laboratory examination

A) Smear preparation

B) Curbol fuchsin stained

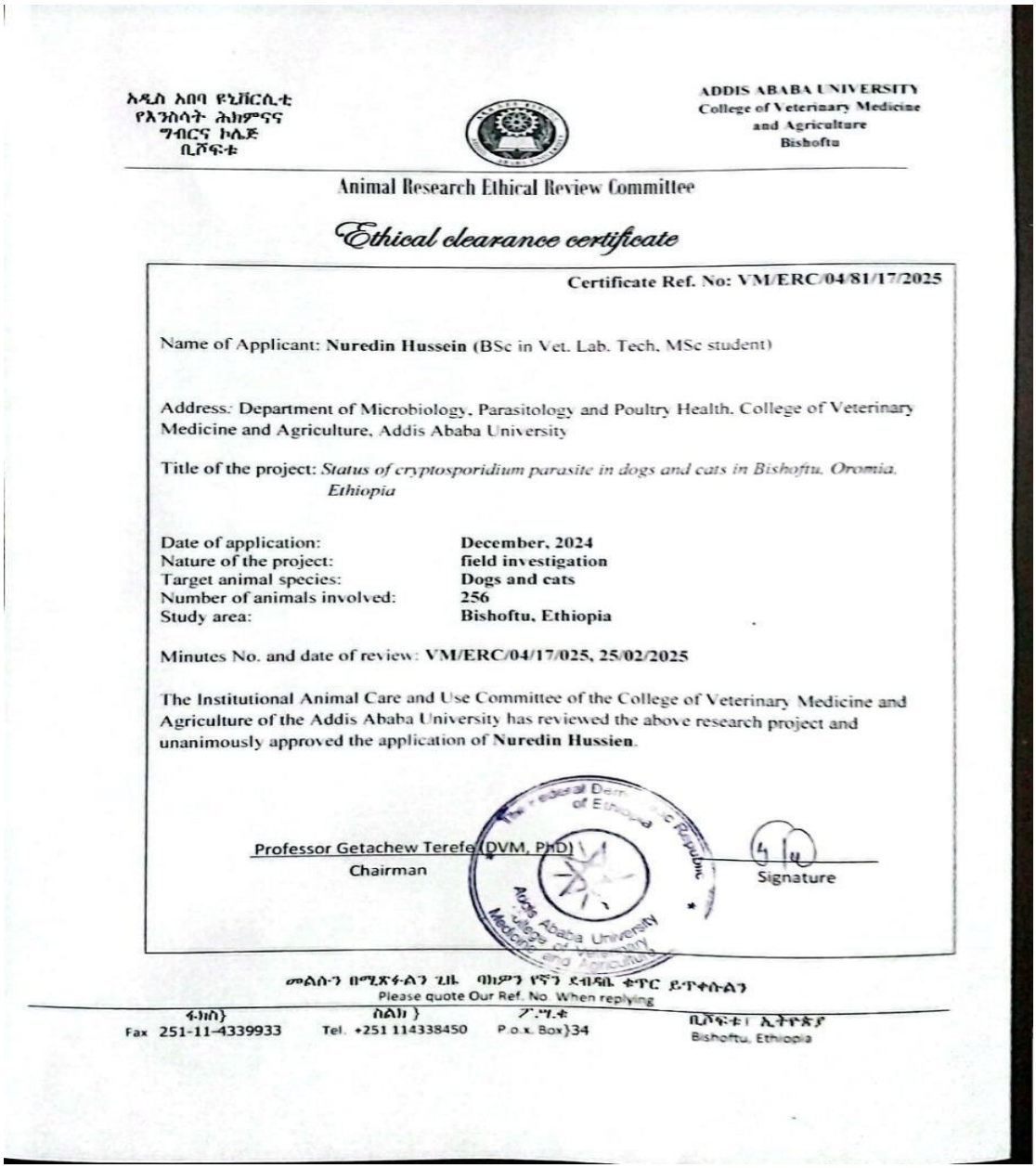
C) methylene blue stained





D) Cryptosporidium result

ANNEX IV Ethical clearance



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ADDIS ABABA UNIVERSITY
College of Veterinary Medicine
and Agriculture
Bishoftu

Animal Research Ethical Review Committee

Ethical clearance certificate

Certificate Ref. No: VM/ERC/04/81/17/2025

Name of Applicant: **Nuredin Hussein** (BSc in Vet. Lab. Tech, MSc student)

Address: Department of Microbiology, Parasitology and Poultry Health, College of Veterinary Medicine and Agriculture, Addis Ababa University

Title of the project: *Status of cryptosporidium parasite in dogs and cats in Bishoftu, Oromia, Ethiopia*

Date of application:	**December, 2024**
Nature of the project:	**field investigation**
Target animal species:	**Dogs and cats**
Number of animals involved:	**256**
Study area:	**Bishoftu, Ethiopia**

Minutes No. and date of review: **VM/ERC/04/17/025, 25/02/2025**

The Institutional Animal Care and Use Committee of the College of Veterinary Medicine and Agriculture of the Addis Ababa University has reviewed the above research project and unanimously approved the application of **Nuredin Hussien**.

Professor Getachew Terefe (DVM, PhD)
Chairman

Signature

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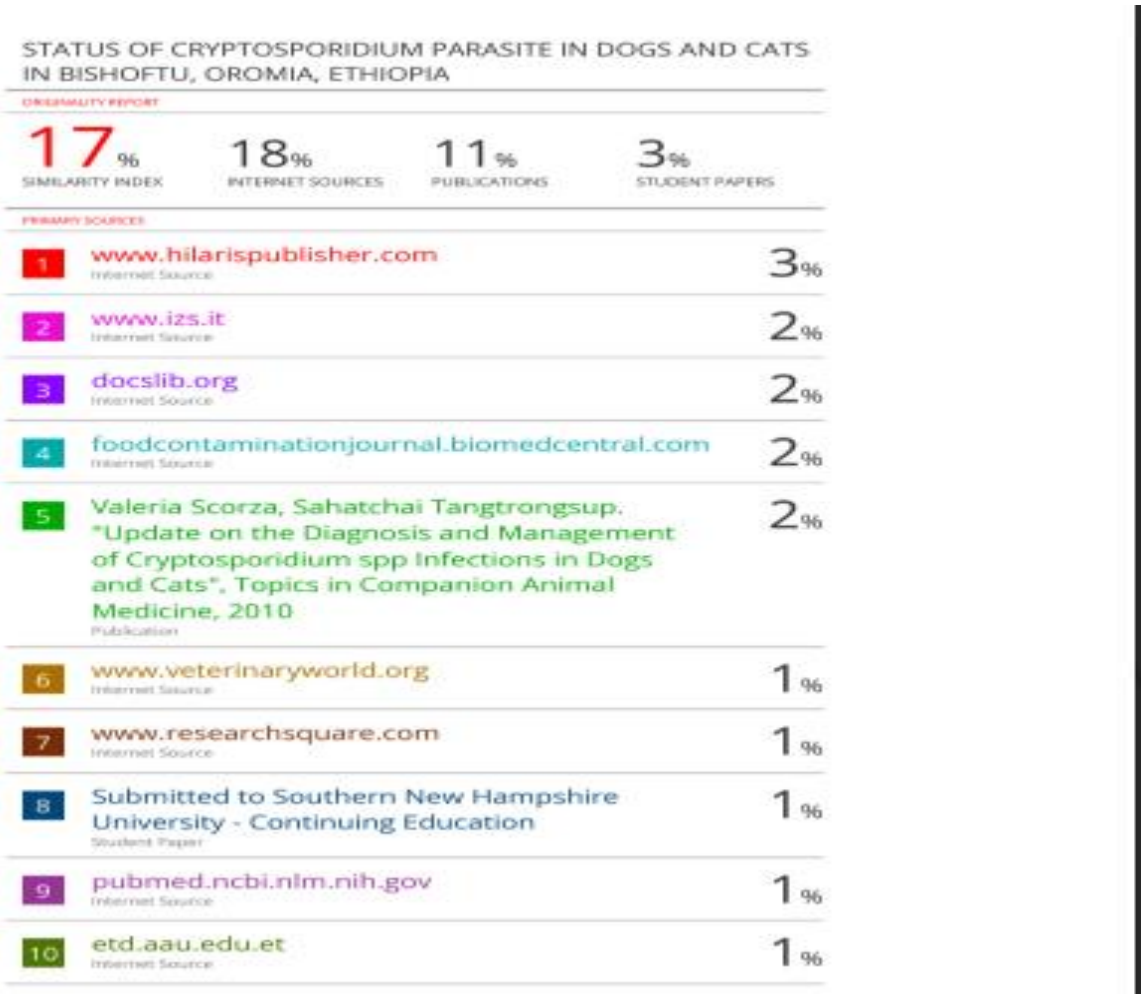
ANNEX V : Plagiarism Check Report



STATUS OF CRYPTOSPORIDIUM PARASITE IN DOGS AND CATS IN BISHOFTU, OROMIA, ETHIOPIA

ORIGINALITY REPORT

17%	18%	11%	3%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

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