WOLKITE UNIVERSITY

COLLEGE OF MEDICINE AND HEALTH SCIENCES

SCHOOL OF GRADUATE STUDIES

CO-INFECTION OF ENTERIC PROTOZOA AND BACTERIA, AND ITS ASSOCIATED FACTORS AMONG UNDER-FIVE DIARRHEIC CHILDREN VISITING WORABE HOSPITAL, CENTRAL ETHIOPIA

MSC THESIS

BY

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Dec, 2024

WOLKITE, ETHIOPIA

Wolkite University

School of Graduate Studies

Co-infection of Enteric Protozoa and Bacteria, and its Associated Factors Among Under Five Diarrheic Children Visiting Worabe Hospital, Central Ethiopia

A Thesis Submitted to the Department of Medical Laboratory Science, College of Medicine and Health Science, in Partial Fulfillment of the Requirements for Master's Degree in Medical Parasitology

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We hereby certify that we have read and evaluated this thesis titled "Co-infection Rate of Enteric Protozoa and Bacteria, and Its Associated Factors among Under Five Diarrheic Children Visiting Worabe Hospital, Central Ethiopia"**.** Prepared under our guidance by Abdirsemed Bedewi Hussen. We recommend that the Thesis shall be submitted as fulfilling the requirements for the award of an MSc Degree in Medical Parasitology.

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As members of the Board of Examiners of the Master of Science Thesis open defense examination, we have read and evaluated this Thesis Prepared by Abdirsemed Bedewi Hussen and examined the candidate. We hereby certify that the thesis is accepted for fulfilling the requirements for the award of the degree of Master of Science (M.Sc.) in Medical Parasitology.

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ACKNOWLEDGMENT

I would like to extend my heartfelt gratitude to Wolkite University College of Health and Medical Science, Department of Medical Laboratory Science, for providing me with the opportunity to pursue this postgraduate study. I am also deeply thankful to Worabe Comprehensive Specialized Hospital for their generous financial support, which made this research possible.

I would like to express my sincere appreciation to my advisors, M/r Lemma Workineh and M/r Gizachew Ayele, for their invaluable intellectual guidance, unwavering support, and leadership throughout my thesis work. Additionally, I am grateful to my data collectors for their dedicated efforts in gathering the data.

Finally, I extend my profound gratitude to the study participants for their voluntary involvement, as well as to all the staff at Worabe Comprehensive Specialized Hospital for their essential assistance during the data collection process.

ABBREVIATIONS AND ACRONYMS

CLSI	=	Clinical and Laboratory Standard Institute
LDC	=	Lysine Decarboxylation
HIPPI	=	Human Intestinal Protozoan Parasitic Infections
LICS	=	Low-Income Countries
MDR	=	Multi-Drug Resistance
MHA	=	Mueller-Hinton Agar
NCCLS	=	National Committee on Clinical Laboratory Standards
ORS	=	Oral Rehydration Salt
PCR	=	Polymerase Chain Reaction
SS	=	Salmonella Shigella Agar
TSI	=	Triple Sugar Agar
XLD	=	Xylose Lysine Deoxycholate

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ABSTRACT

Background: *Diarrheal diseases remain a leading cause of illness and death among children under five in developing countries, including Ethiopia. Enteric pathogens such as Giardia lamblia, Entamoeba histolytica, Salmonella, and Shigella spp. are common causes of childhood diarrhea in these regions. However, data on their sources and co-infection patterns were limited.*

Objective*: This study aimed to assess the co-infection rates of enteric protozoa and bacteria and identify associated risk factors in children under five presenting with diarrhea at Worabe Hospital, central Ethiopia.*

Method*: A cross-sectional study was conducted from February to April 2024. Stool samples were collected from 250 children under five with diarrhea and analyzed using parasitological and bacteriological techniques to detect the presence of pathogens.*

Results*: Out of 250 stool samples analyzed, 72 (28.7%) tested infected for intestinal protozoa, and 33 (13.2%) were infected for enteropathogenic bacteria. Co-infections were identified in 20 cases (8.0%). The most frequent co-infections included G. lamblia with Salmonella spp. (2.8%), G. lamblia with Shigella spp. (2.0%), E. histolytica/dispar with Shigella spp. (2.4%), and E. histolytica/dispar with Salmonella spp. (0.8%). Factors significantly associated with co-infection included large family size (p=0.042), guardians with untrimmed fingernails (p=0.013), not washing vegetables before feeding children (p=0.003), guardians not washing hands before feeding children (p=0.02), presence of domestic animals in the house (p=0.017), lack of a home latrine (p=0.001), and use of unprotected drinking water (p=0.004).*

Conclusions and Recommendations*: The study highlights that intestinal protozoa and enteric bacteria, as well as co-infections, are major contributors to diarrhea in children. Key risk factors include poor personal hygiene, untrimmed fingernails, and inadequate environmental sanitation. It is recommended that public health efforts focus on raising awareness about personal and environmental hygiene to reduce the burden of diarrheal diseases.*

Keywords: *Co-infection, enteric protozoa, bacteria, under-five children, diarrhea, Worabe Hospital, Ethiopia.*

1. INTRODUCTION

1.1. Background

The passage of three or more loose or watery stools in 24 hours is known as diarrhea, and it can be either infectious or non-infectious (1). Among children under five, it is a major source of illness and mortality, particularly in countries with low and middle incomes (2). Multiple enteric pathogen infections, especially co-infections with intestinal protozoa and bacteria, are a major contributing cause to the high frequency of diarrhea in this age group (3).

Intestinal protozoa and bacteria co-infection vary according to environmental, socioeconomic, and health-related factors. The main causes of children's increased risk of co-infections include inadequate healthcare facilities, poor sanitation, and limited access to clean water. Furthermore, nutritional state, overcrowding, and household cleanliness habits can affect severity and susceptibility (4,5). These circumstances create the framework for the ongoing spread of parasitic intestinal protozoa and bacterial infections.

Intestinal protozoa are microscopic, single-celled organisms that inhabit the digestive systems of both humans and other animals (6). These protozoans play diverse roles, ranging from being harmless commensals to causing significant diseases. They are primarily classified into two groups: non-pathogenic species that coexist with their host without causing harm, and pathogenic species that can lead to severe gastrointestinal illnesses (7).

Pathogenic intestinal protozoa, such as *Entamoeba histolytica*, *Giardia lamblia*, and *Cryptosporidium spp.*, are of particular concern in public health (8). Amoebiasis, giardiasis, and cryptosporidiosis are among the illnesses they cause, and their symptoms can range from moderate diarrhea to severe, potentially fatal dysentery. The usual way that these illnesses are transmitted is by consuming tainted food or drink (9).

E. histolytica infections are most common in adults, but they can also be found in youngsters. Because the parasite affects the large intestine, severe infection causes significant blood loss and may also cause systemic issues such as liver abscesses caused by parasite infection of the liver.

Amoebiasis can induce nutritional loss and reduced protein levels in the blood, which can contribute to malnutrition (10). *G. lamblia* infections damage the intestinal mucosa, resulting in nutritional malabsorption, particularly of fat. It appears to be common in children suffering from malnutrition, resulting in decreased growth and weight loss (11).

Cryptosporidium parvum impairs mucosal morphology, which can lead to nutrient loss and malnutrition. Infected individuals shed the parasite in their feces in the form of oocysts as early as 7 days after infection and for up to 5 weeks after the diarrheal disease has ended (12).

Diarrhea symptoms can vary depending on the pathogen. For example, *G. lamblia* often causes watery, foul-smelling diarrhea, while dysentery is more commonly linked to *E. histolytica* (13). Microscopic identification of parasites remains the gold standard for diagnosis, but its accuracy depends on the skill of the examiner. Therefore, alternative diagnostic methods such as enzyme immunoassays, direct fluorescent-antibody tests, and PCR are increasingly important for improving diagnostic sensitivity (14,15).

Intestinal protozoan parasites are now prevented by stopping the infectious cyst stage of the parasite's fecal-oral transmission. Because cysts are resistant to iodine and chlorine, raw vegetables must be washed with soap and steeped in vinegar for 15 minutes before being consumed, and water in developing countries must be boiled before drinking. Screening for intestinal *G. lamblia* and *E. histolytica* in the family members of an index case is crucial to halting the spread of protozoan diseases (16). Safe disposal of human and animal waste, improved environmental and personal hygiene, proper use of latrines, and early diagnosis and treatment are the main strategies for avoiding and managing protozoan parasite diseases (17).

Bacterial infections account for 10–20% of all occurrences of acute gastroenteritis in children, making them the second most common cause of gastrointestinal issues (18). Children of all ages are susceptible to this infection, although toddlers and children under five are most frequently affected. Clinical symptoms including fever and the presence of blood and mucus in the stool are usually indicative of the infection (19).

Based on their modes of infection and illness manifestation, pathogenic bacteria in the stomach can be divided into several types. Some of the most well-known intestinal pathogens include *Escherichia coli* (specifically its pathogenic strains like *EPEC, ETEC, and EHEC*), *Salmonella*, *Shigella*, *Vibrio* cholerae, and *Clostridioides difficile* (20). These pathogens employ a range of strategies to invade and colonize the host intestinal tract, such as producing toxins, adhering to intestinal cells, and evading the host immune system (21).

The fecal-oral pathway is usually how these bacteria are spread, frequently through tainted food, water, or close contact with infected people. Depending on the bacterial species, the infectious dose, and the host's immune condition, infection symptoms might range from minor gastrointestinal discomfort to severe dehydration and systemic consequences (22).

The gold standard for determining the etiologic diagnosis of enterobacteria is still conventional bacterial stool culture. Acute diarrheal symptoms can be treated with exclusive breastfeeding for six months, infrastructural improvements to guarantee basic sanitation and clean drinking water, health education focused on hygiene practices, and vaccinations (23). Acceptable waste treatment and hand washing after handling waste are especially crucial in shigellosis prevention due to their high transmissibility and the fact that person-to-person is the primary channel of impurity (24). In addition, the separation processing of food stuffs of animals and factory foods vegetables fruits is well done to avoid cross-contamination (25).

Childhood diarrhea may be associated with concurrent infections of intestinal protozoa and enteropathogenic bacteria (26). As previously stated, it is difficult to link gastroenteritis to a specific etiological agent, especially as individuals who are co-infected by many groups of pathogens are generally thought to be in a lower condition than people who have single infections. As a result, co-infections increase the likelihood of clinical complications and treatment costs, and studies to investigate the role of co-infections with enteric pathogens in people with acute diarrhea are urgently needed (27). To improve the management of severe diarrhea in children, global health organizations should prioritize an understanding of the role of co-infection with enteric pathogens (protozoa and bacteria) (28).

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1.2. Statement of the problem

Globally, diarrheal illnesses continue to be a serious public health concern, especially for young children (under five). About 15% of pediatric fatalities globally are caused by them, with rates rising to 25% in Africa and 31% in Southeast Asia, making them one of the main causes of morbidity and mortality. Diarrhea is the second leading cause of illness and mortality among children in Sub-Saharan Africa (29). Notably, certain nations have shockingly high rates of pediatric mortality, with numbers like 133 deaths for every 1,000 live births in Chad and 104 in the Democratic Republic of the Congo (30).

In Ethiopia, 12% of pediatric patients reported having diarrhea in the two weeks before the 2016 Demographic and Health Survey, making it the country's second most common cause of death for children (31). Ethiopian children had a 22% pooled prevalence of diarrhea, according to a review of publications. The Afar area had the highest rates (27%) and Somalia, Dire Dawa, and Addis Ababa had the greatest incidences (32).

In Ethiopia, diarrhea is the leading cause of illness and mortality among children, and it is made worse by inadequate healthcare facilities, a lack of clean water, and poor sanitation. Enteric protozoa and bacteria are the most common etiological agents of diarrheal episodes (33). However, co-infections with both protozoa and bacteria are frequently underreported and poorly understood, despite their potential to complicate clinical outcomes and challenge treatment regimens.

The co-infection of enteric protozoa and bacteria in diarrheic children can lead to more severe and prolonged illnesses, posing a greater risk of malnutrition, dehydration, and mortality (34). Understanding the prevalence and associated factors of such co-infections is crucial for improving diagnostic accuracy, optimizing treatment regimens, and implementing targeted public health interventions.

Worabe Hospital, located in Central Ethiopia, serves a population facing significant healthcare challenges, including high rates of childhood diarrheal diseases. However, there is a lack of comprehensive data on the co-infection of enteric protozoa and bacteria among under-five diarrheic children in this region. Identifying the burden of co-infections and their associated risk factors can provide critical insights for healthcare providers to enhance the management and prevention of diarrheal diseases in this vulnerable population.

To close the gap, this study will measure the co-infection between bacteria and enteric protozoa and identify the risk factors for these infections in children under five who are visiting Worabe Hospital with diarrhea.

1.3. Objectives of the Study

1.3.1. General Objective

- To assess the co-infection of intestinal protozoa and enteropathogenic bacteria, along with the associated factors, among under-five children with diarrhea who visited Worabe Hospital, Central Ethiopia.

1.3.2. Specific Objectives

- To determine the prevalence of intestinal protozoa infections in under-five children with diarrhea.
- To determine the prevalence of enteropathogenic bacterial infections in under-five children with diarrhea.
- To assess the co-infection between intestinal protozoa and enteropathogenic bacteria in under-five children with diarrhea.
- To identify the factors associated with intestinal protozoa and enteropathogenic bacterial infections in under-five children with diarrhea.

1.4. Research Questions

1. What is the prevalence of intestinal protozoa infections among under-five children with diarrhea?

2. What is the prevalence of enteropathogenic bacterial infections in under-five children with diarrhea?
3. What is co-infection between intestinal protozoa and enteropathogenic bacteria in under-five children with diarrhea?
4. What are the factors associated with intestinal protozoa and enteropathogenic bacterial infections among under-five children with diarrhea?

1.5. Significance of the study

This study examined the co-infection of intestinal protozoa and enteropathogenic bacteria, as well as the associated factors, among under-five diarrheic children visiting Worabe Comprehensive Specialized Hospital. The findings provided crucial information for health authorities and stakeholders to develop effective prevention and control strategies for diarrheal diseases in young children.

By shedding light on the prevalence of these infections, the study served as an important resource for health bureaus to enhance health promotion activities. The insights gained can be guided in reducing the burden of intestinal protozoa and enteropathogenic bacteria infections. Additionally, it will fill existing knowledge gaps and provide valuable data for future research in this area.

The study also has the potential to improve community health by promoting better hygiene practices and raising awareness about how these infections spread. Ultimately, it contributed to improving health outcomes for children by facilitating timely diagnosis and treatment for those harboring intestinal protozoa and enteropathogenic bacteria.

2. LITERATURE REVIEW

2.1. Diarrhea Diseases

Gastrointestinal tract illnesses that are brought on by bacteria, viruses, or parasites are indicated by diarrhea. These illnesses are usually contracted through contaminated food, water, or human-to-human contact, frequently as a result of inadequate personal hygiene. A severe type of diarrhea called dysentery is characterized by mucus and blood in the stool. Diarrhea is categorized as chronic if it persists for more than 14 days, persistent if it lasts for more than seven days, and acute if it lasts for less than seven days (35). It has been demonstrated that effective preventive measures, such as regular hand washing with soap, better sanitation, and access to clean drinking water, greatly lower the incidence of diarrheal illnesses (36).

2.2. Prevalence of intestinal protozoa infection

Diarrheal illnesses are largely caused by intestinal protozoan infections, especially in children under five. According to a cross-sectional study on diarrhea in children ages 0–168 months carried out in Mozambique between June 2014 and January 2018, 21% (212/1008) of the samples had at least one parasite infection. The most common was *G. lamblia* (9.7%), which was followed by *E. histolytica* (2%) (9). Another study conducted in Dares Salaam, Tanzania, looked at 720 stool samples from children under five who were admitted with either acute or chronic diarrhea. The most often discovered protozoa were *G. lamblia* (35.6%) and *E. histolytica* (12.2%) (37).

In Yamoussoukro, Côte d'Ivoire, a study on 306 stool samples revealed that 62.75% of the samples were positive for at least one protozoan parasite, with *G. lamblia* and *E. histolytica* showing frequencies of 20.92% and 22.55%, respectively (38). A study from Bahir Dar, Ethiopia, conducted between November 2011 and February 2012, found a 20.4% prevalence of intestinal protozoan infections among 422 children, with *E. histolytica /dispar* (15.2%) and *G. lamblia* (3.8%) being the most common parasites (39).

In Zeita Primary School in Central Ethiopia, a more recent cross-sectional survey carried out between January and March 2020 revealed that 46.8% of the 280 children there had intestinal protozoan parasite infections. *E. histolytica* (25.2%) and *G. lamblia* (19.3%) were the most commonly found protozoa (8). Another retrospective study from Atat Hospital in Ethiopia found that intestinal protozoan parasites were present in 45% of 15,731 stool samples. Of these, 60% were caused by *E. histolytica*, while the lowest incidence was 3.6% caused by *G. lamblia* (40).

From February 2011 to August, Yirgalem Hospital carried out a case-control research to ascertain the prevalence of *G. lamblia* and *E. histolytica/dispar* in children with diarrhea. At least one intestinal parasite was detected in 84 (36.52%) of the children, 57 (49.56%) of whom had diarrhea and 27 (23.47%) of whom did not (22).

From April to October 2019, a cross-sectional study was carried out in Eastern Tigrai, Ethiopia, among children who had diarrhea in Adigrat's medical facilities. The total frequency of protozoan infection among 418 children with diarrhea was 59.3% (248/418) (41).

2.3. Prevalence of enteropathogenic bacteria

Enteropathogenic bacteria are key contributors to diarrheal diseases. A study conducted in China from 2014 to 2017 identified *Salmonella* as the leading bacterial pathogen, with 843 isolates collected from diarrheic patients, including 67 different serotypes (42). In Libya, *Salmonella* was found to be the primary cause of childhood diarrhea, while research in Mozambique also highlighted *Salmonella* and *Shigella* as the most common enteropathogenic bacteria associated with diarrhea (43).

A prospective study conducted in the Central Republic of Africa between July 2011 and June 2012 on 2,500 pediatric patients reported 182 cases of *Shigella* and 89 cases of *Salmonella* (44). In Ethiopia, a cross-sectional study on 290 children with diarrhea at health facilities in Addis Ababa revealed *Shigella* species in 7.6% of cases, followed by *Salmonella* in 2.4% (45).

According to a facility-based cross-sectional study carried out on 263 pediatric patients with diarrhea who were selected using a practical sampling method between April 2018 and July 2019 in southern Ethiopia, 8% (21/263) *Shigella* and *Salmonella* were isolated (46). At the Debre Berhan Referral Hospital in Ethiopia, 163 children under five who had acute diarrhea had their enteropathogens examined using a cross-sectional study design centered at a health facility. Of the subjects, 55.8% (91/163) had enteropathogens. The bacterial culture-positive rate was 46% (75/163) (47). Another cross-sectional study was conducted in Ambo town, Ethiopia, between January and July 2014, involving 239 under-five children who had diarrhea. Only nine (3.8%) of the 239 children who were checked tested positive for *Shigella* (n = 6) or *Salmonella* (n = 3) (48).

2.4. Co-infection of intestinal protozoa, and enteropathogenic bacteria

Co-infection with protozoa and bacteria can complicate diagnosis and treatment. A study from South Africa conducted between June 2014 and June 2016 on 184 stool samples found that 78% of samples were positive for one or more pathogens, and 55% of these exhibited multiple co-infections. Notably, co-infection with bacteria and protozoa was observed in 12.5% of the samples, with *Shigella* and *G. lamblia* co-infections detected in 2.1% of cases (49). Another study conducted from May 2016 to February 2018 on 132 children under five years old found that 46.1% of stool samples tested positive for diarrheal pathogens, with 29.4% of these cases involving multiple infections, including *Salmonella* and *G. lamblia* (50).

Research in Addis Ababa, Ethiopia, showed that co-infections involving *Salmonella* were frequently associated with *E. histolytica* (9.7%) and *G. lamblia* (4.8%) (28). These findings suggest that co-infections are prevalent and pose additional challenges to the management of diarrheal diseases.

2.5. Associated factors of intestinal protozoa and enteropathogenic bacteria

Several studies have identified socio-economic and environmental factors that contribute to the risk of protozoan and bacterial infections. Poverty, poor hygiene, lack of access to clean water, open defecation, and low education levels are key factors linked to higher rates of diarrheal diseases (21). A study in southeastern Iran found a significant association between intestinal parasitic infections and factors such as drinking water sources and residency status (51). In Ethiopia, a systematic review revealed that factors like family size, open defecation, hand-washing habits, and raw vegetable consumption were strongly associated with intestinal protozoan infections (17).

A cross-sectional study conducted in Zeita village, Merhabete District, North Shewa Zone, Central Ethiopia, found that various Sociodemographic factors, including family occupation, drinking water sources, water handling practices, vegetable consumption habits, and the presence of latrines, were significantly associated with the occurrence of intestinal protozoan parasites (52).

In a March–May 2017 study of 167 diarrheal children under five in three government health facilities in Arba Minch town, multivariable logistic regression analysis showed that families making less than 1,000 Ethiopian Birr per month were 6.5 times more likely to have children infected with enteric pathogens than families making more than 1,000 Birr (53,54).

Shigella infections were more common in children with less education, according to a different study done on 272 pediatric patients with diarrhea in Gondar Town healthcare facilities between January and March 2018. However, children whose mothers had completed secondary or higher education had a lower frequency of *Shigella*. Between August and November 2009, a study conducted at Felege Hiwot Referral Hospital in North West Ethiopia also found a strong correlation between the prevalence of *Shigella* and educational attainment (55,56).

The study in Debremarkos town also found that urban children were seven times more likely to contract *Salmonella* or *Shigella* infections than their rural counterparts (22). A study conducted at Altamura Health Center in Southern Ethiopia from April 2018 to July 2019 identified that infrequent hand washing after toilet use and storing food on open dishes were significantly associated with *Salmonella* and *Shigella* infections (57).

According to a study done in Gondar town between January and March 2018, children who ate raw food were 3.7 times more likely to get *Shigella* (58 infections). Another study carried out at HFSUH in Eastern Ethiopia between February and May 2011, discovered that the lack of latrines and the use of water from unprotected sources were important risk factors for *Shigella* infection (59).

Furthermore, the Arba Minch study found that poor hand hygiene practices before meals increased the chance of contracting *Shigella* or *Salmonella* illnesses by a factor of six.

Additionally, compared to kids with trimmed fingernails, kids with untrimmed fingernails were four times more likely to get these illnesses (60)

2.5. Conceptual Framework

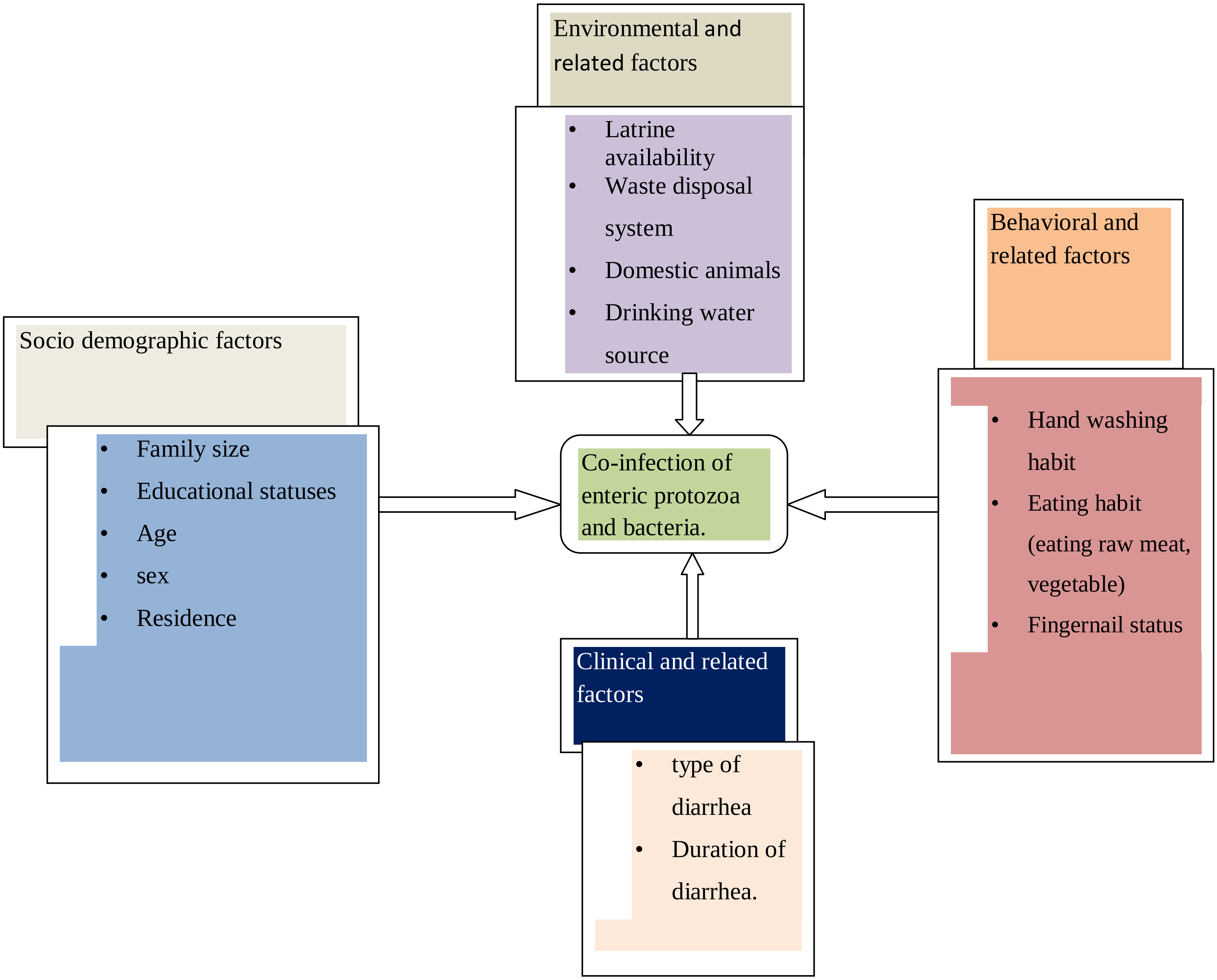

Figure 1: a conceptual framework that shows the linkage of intestinal protozoa and enteropathogenic bacteria with different factors

3. METHODS AND MATERIALS

3.1. Study area

This study was conducted at Worabe Comprehensive Specialized Hospital, located in the Silte Zone of Central Ethiopia. Worabe town, the administrative center of Silte Zone, is situated approximately 172 kilometers south of Addis Ababa, the capital city of Ethiopia. The hospital serves as a major healthcare facility for the zone and its surrounding areas, providing specialized medical services to a predominantly rural population.

The region is characterized by a tropical climate with distinct wet and dry seasons and sits at an elevation of approximately 2,128 meters (6,982 feet) above sea level (61). This contributes to the transmission dynamics of various infectious diseases, including diarrheal diseases caused by enteric pathogens. The community in the area faces challenges such as limited access to clean drinking water, poor sanitation, and inadequate healthcare infrastructure, all of which are known risk factors for diarrheal diseases. These conditions make Worabe an important location for studying the co-infection of enteric protozoa and bacteria, particularly in vulnerable populations such as children under five.

Worabe Comprehensive Specialized Hospital is equipped with diagnostic and treatment facilities for managing infectious diseases, making it a suitable setting for investigating the prevalence and risk factors associated with co-infections among under-five diarrheic children. The hospital also serves as a referral center for patients from nearby districts, allowing for a diverse study population and providing valuable data on the regional burden of diarrheal diseases.

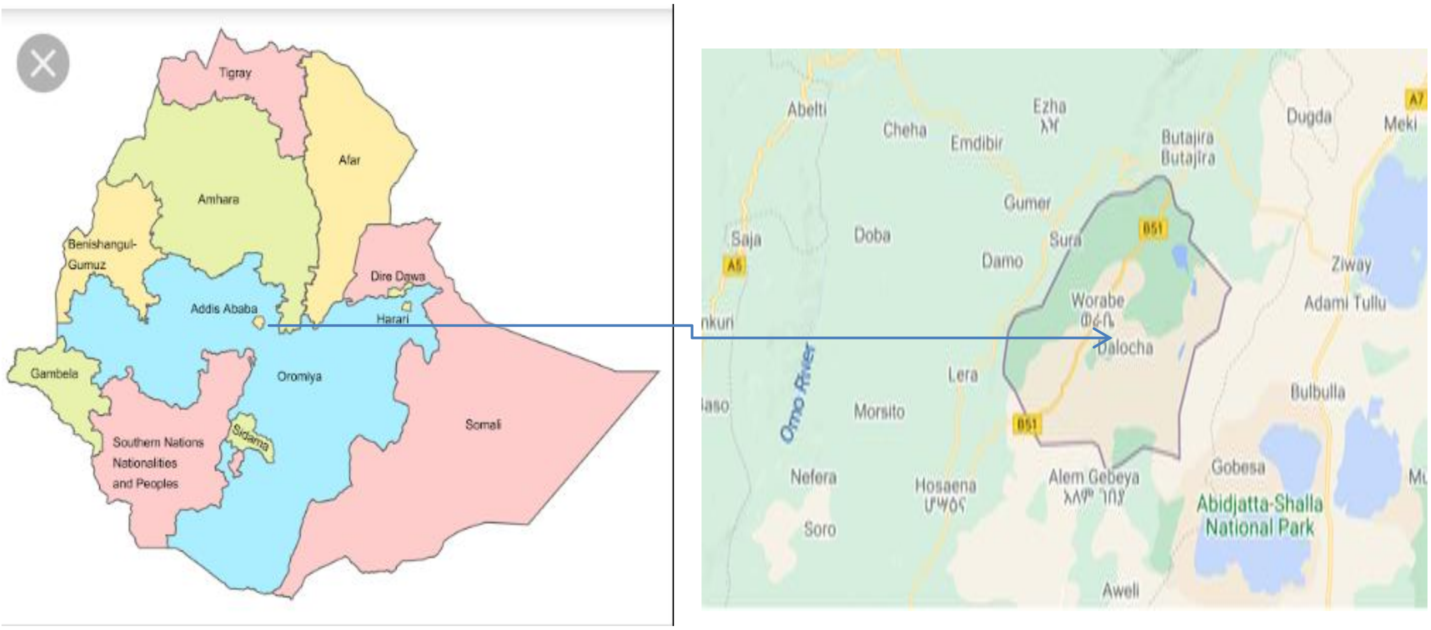

Figure 2: Map of the study area, source Google

3.2. Study Design and Duration

This study was designed as a cross-sectional survey and took place over three months, from February to April 2024.

3.3. Study Population

3.3.1. Source populations

- ✓ All under five children visiting Worabe Comprehensive Specialized Hospital

3.3.2. Study populations

- ✓ All under five diarrheic children visiting Worabe comprehensive specialized hospitals within the study period

3.4. Eligibility criteria

3.4.1. Inclusion Criteria

- ✓ Diarrheic under-five children visiting Worabe Comprehensive Specialized Hospital within the study period

3.4.2. Exclusion criteria

- ✓ Children on antibiotic treatment during data collection time and children who received anti-protozoa drugs within two weeks before the data collection date (62).
- ✓ Under-five Pediatric patients with diarrhea who were unable to give stool samples.
- ✓ Lack of parental Asent

3.5. Variables of the Study

3.5.1. Dependent Variable

- Co-infection of enteric protozoa and bacteria

3.5.2. Independent Variables

1. Socio-Demographic Factors

- Age
- Sex
- Residence
- The educational level of guardians
- Occupational status

2. Clinical Data

- History of previous diarrhea episodes
- Type and duration of diarrhea
- Associated signs and symptoms

3. Environmental Factors

- Source of water and food
- Waste disposal methods
- Availability of latrines

4. Behavioral Factors

- Personal hygiene practices, such as handwashing and feeding habits
- Fingernail cleanliness

3.6. Sample Size Determination

For the first objective, the sample size was calculated using the single population proportion formula. The prevalence rate used was based on a previous study on the prevalence of diarrhea-causing protozoan infections in under-five children with diarrhea in Bahir Dar town, northwest Ethiopia, which reported a prevalence of 20.5% (39). The confidence level was set at 95%, with a margin of error of 5%. The formula used for calculation is as follows:

$n = [(Z\alpha/_2)^2\ p\ (1\text{-}p)]/\ d^2$

Where:

n is the sample size

p is the prevalence of intestinal protozoa (0.205)

$q=1-p$

$d2$ is the square of the margin of error (0.05)

$Z\alpha/2=1.96$ for a 95% confidence level

Plugging in the values:

$$n = \frac{(1.96)2\ (0.205)\ (1\text{-}0.205)}{(0.05)2} = 250$$

For the second objective, the sample size was determined using the same formula, but this time considering the prevalence of diarrhea-causing bacteria in under-five children from a study conducted in Ambo town, western Ethiopia, which reported a prevalence of 18% (48). The same 95% confidence level and 5% margin of error were used.

$n = [(Z\alpha/_2)^2\ p\ (1\text{-}p)]/\ d^2$

Where:

P is the prevalence of Salmonella and Shigella spp. (0.18)

$q = 1 - p$

$d2$ is the square of the margin of error (0.05)

$Z\alpha/2 = 1.96$ for a 95% confidence level

Plugging in the values:

$$n = \frac{(1.96)2\ (0.18)\ (1-0.18)}{(0.05)2} = 226$$

In summary, the final sample size for the study was determined to be 250, as it is the larger of the two calculated sample sizes.

3.7. Sampling Techniques

A convenient sampling method was used, where all consecutive patients who met the inclusion criteria were included in the study.

3.8. Data collection

3.8.1. Data Collectors

The data were gathered by a team consisting of two clinical nurses and two medical laboratory professionals, under the supervision of two experienced senior medical laboratory professionals.

3.8.2. Questionnaire Data

To collect the necessary data, a structured questionnaire was developed by reviewing existing studies and relevant literature. Initially drafted in English, the questionnaire was later translated into Amharic to ensure clarity and accuracy. It was designed to capture a wide range of factors, including socio-demographic, behavioral, environmental, and clinical aspects. Data was gathered through face-to-face interviews with participants or their guardians, where trained clinical nurses conducted physical exams and reviewed pediatric patients' medical histories, including treatment records.

The questionnaire addressed various socio-demographic details, such as age, gender, residence, education, occupation, and income. Clinical factors were also explored, including the history of diarrhea, its type and duration, related symptoms, and any treatments received. Additionally, behavioral factors like personal hygiene (including hand washing, feeding habits, and fingernail cleanliness) were considered, along with environmental aspects such as waste disposal practices, water sources, hand washing habits, and toilet usage.

3.8.3. Laboratory Procedures

3.8.3.1. Stool Sample Collection

Stool specimens were collected following standard operating procedures. After gathering essential participant data, 5 grams of stool were collected in sterile containers from 250 pediatric patients suffering from diarrhea. Each vial was labeled with the participant's code number. Participants were instructed to avoid mixing the stool sample with urine or other contaminants during collection.

3.8.3.2. Stool Sample for Parasitological Examination

3.8.3.3. Wet Mount Method

A drop of physiological saline and a drop of Lugol's iodine were placed at opposite ends of a glass slide, which was labeled with the participant's ID number. Using a wooden applicator stick, a small sample of stool, about the size of a match head, was carefully mixed with the saline and iodine to form a thin, even suspension. Great care was taken to ensure the mixture wasn't too thick, which could hide organisms, nor too thin, which might leave gaps on the slide. The suspension was then covered with a cover slip, making sure no air bubbles were trapped. The slide was examined under a microscope at low power, with the entire coverslip carefully scanned for signs of parasitic stages, such as trophozoites, cysts, and ova (14).

3.8.3.4. Formal-Ether Concentration Method

About 2 grams of stool were carefully placed into a centrifuge tube containing 10 mL of 5-10% formalin, using an applicator stick. Once the stool was mixed with the formalin, the resulting mixture was filtered through a nylon filter into a test tube. To remove any solid residues, the filtrate was washed with normal saline. Next, the mixture was transferred to a tube with 7 mL of ethyl acetate, which was sealed and shaken vigorously. After this, the tube was centrifuged for 2 minutes. The sample was then allowed to stand for 5 minutes, during which four distinct layers formed: a top layer of ether, a debris plug, a clear formalin layer, and sediment at the bottom. The debris plug was gently removed with a cotton swab, and the liquid was discarded, leaving behind a small amount of formalin with the sediment. The sediment was then transferred to a slide, covered with a cover slip, and examined under both 10x and 40x magnification to look for cysts and ova. A second slide was prepared by adding a drop of iodine solution for further analysis (14).

3.8.3.5. Modified Ziehl-Neelsen Method for Detecting Intestinal Coccidian

To detect intestinal coccidian, the modified Ziehl-Neelsen staining method was used. First, two thin smears were made from both fresh stool samples and concentrated stool sediments. After allowing the smears to air-dry, they were fixed with methanol for 5 minutes. The slides were then stained with carbol fuchsine for 30 minutes before being rinsed with tap water. To decolorize, acid-alcohol was applied for 1-3 minutes, followed by another rinse with tap water. Next, the slides were counterstained with methylene blue for 1 minute and rinsed again. After drying, the slides were examined under a light microscope at 100x magnification (14).

3.8.3.6. Bacterial Isolation and Identification

To isolate and identify bacteria from stool samples, the process begins with inoculating the samples onto MacConkey agar using a sterile wire loop. These plates are then incubated at 37°C for 24 hours under aerobic conditions. Colonies that appear colorless or pale, indicating they are non-lactose fermenting, are transferred to Xylose Lysine Deoxycholate (XLD) agar and

Salmonella Shigella (SS) agar for further analysis. These plates are also incubated at 37°C for another 24 hours.

On XLD agar, pink to red colonies, and SS agar, colorless colonies (with or without black centers) are considered potential Salmonella or Shigella. These colonies undergo a series of biochemical tests for confirmation.

The tests include:

Triple Sugar Iron (TSI) Agar: Checks for hydrogen sulfide (H_2S) production, gas formation, and carbohydrate fermentation.

Motility Test: Assesses whether the bacteria can move.

Indole Test: Determines if the bacteria can metabolize tryptophan to indole.

Citrate Utilization Test: Evaluates if citrate can be used as the sole carbon source.

Urea Hydrolysis Test: Detects the presence of urease activity.

Lysine Decarboxylase (LDC) Test: Identifies the ability to decarboxylate lysine.

Mannitol Fermentation Test: Tests for mannitol utilization.

Oxidase Test: Checks for the presence of cytochrome c oxidase (63).

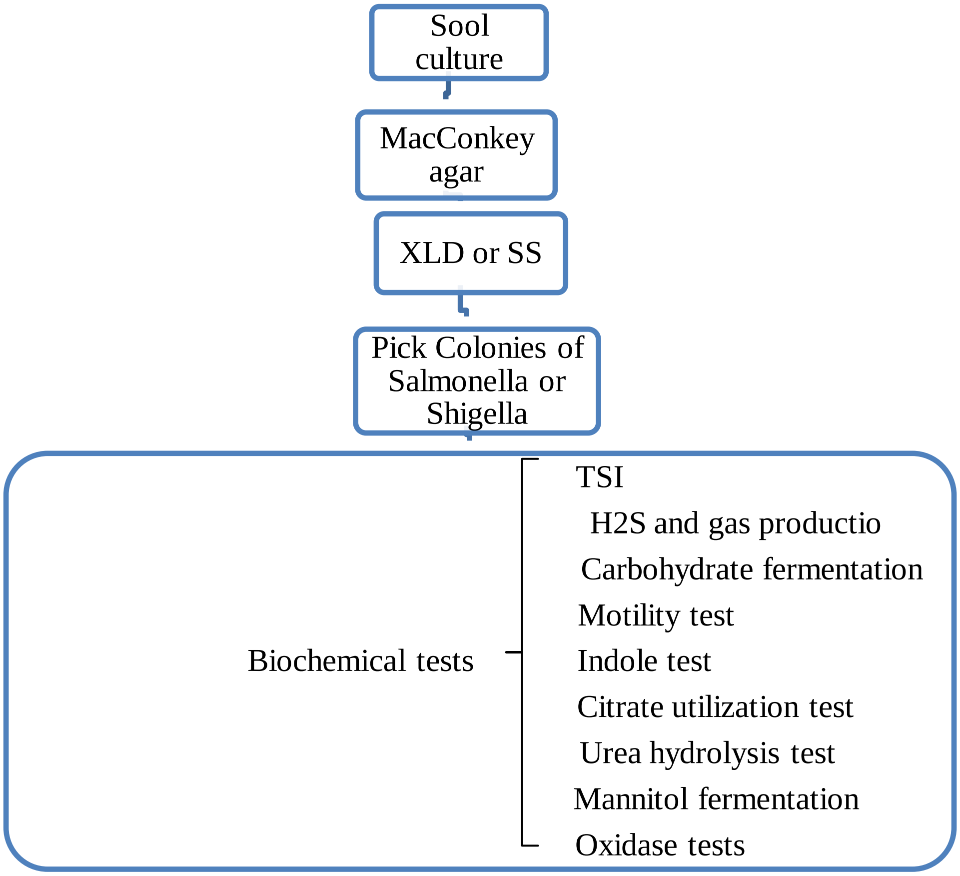

Figure 3: Summary of Procedures for Isolation of *Salmonella* and *Shigella* from Stool Specimen

3.9. Operational Definition

- **Diarrhea**: The passage of three or more loose or liquid stools within 24 hours, or more frequent bowel movements than what is normal for the individual (64).
- **Co-infection:** refers to the simultaneous infection of an individual by two or more distinct pathogens, such as bacteria, viruses, fungi, or parasites, at the same time (65).
- **Hand Hygiene**: A term that refers to cleaning hands either by washing with soap and water or using alcohol-based hand sanitizers without water (66).
- **Fingernail Hygiene**: Maintenance of trimmed fingernails to prevent harboring protozoan cysts and bacteria that may cause infection (66).
- **Protected Drinking Water**: Water obtained from sources that reduce contamination risk, such as treated or boiled water (67).

- **Unprotected Drinking Water**: Water from sources vulnerable to contamination, like rivers, ponds, or open wells (67).
- **Latrine Availability**: Presence or absence of a designated toilet facility in a household, impacting sanitation and hygiene (68).

3.10. Data Quality Assurance

To ensure quality control, all laboratory procedures, including the collection and handling of stool samples, were conducted following established protocols (WHO, 1991). Standard Operating Procedures (SOPs) were followed diligently, and every step was carefully executed. Respondents were provided with instructions on proper stool sample collection to avoid contamination. Appropriate preservatives were used, and prepared slides were stored under suitable conditions.

The questionnaire was pre-tested to ensure the accuracy of the information collected. The sterility of the culture media was verified by incubating a sample of each batch at 37°C overnight. Any batch showing microbial growth was discarded (69).

3.11. Data analysis

The data were coded, cleaned, and entered using Epidata 3.1, and then exported to the Statistical Package for the Social Sciences (SPSS) version 25.0 for further analysis. Descriptive statistics, including frequency distributions, means, medians, and standard deviations, were used to analyze the prevalence of intestinal protozoa and enteropathogenic bacteria and their association with diarrhea. After completing the data entry, the soft copy was cross-checked against the hard copy to ensure consistency. Any missing values, outliers, and inconsistencies were identified and addressed during the data-cleaning process. Subsequently, both bivariate and multivariable logistic regression analyses were conducted to identify associations between dependent and independent variables.

3.12. Ethical Considerations

The study received ethical approval from the Ethical Review Board of the College of Medicine and Health Sciences at Wolkite University. Given that the participants were children under five years old, their parents were thoroughly informed about the study's objectives and purpose. Written consent was obtained from the parents, who were assured that participation was completely voluntary. They had the freedom to decide whether or not their child would take part.

To protect the privacy of participants, all collected data was kept confidential. If laboratory tests revealed the presence of intestinal parasites or harmful bacteria, the findings were promptly shared with physicians so that the affected children could receive appropriate treatment.

3.13. Dissemination plan and use of results

The findings of this study will be disseminated to Wolkite University, College of Medicine and Health Sciences, and the following bodies; the Worabe City Health Department office and also stakeholders who have contributed to strengthening the prevention of pathogenic intestinal protozoa and enter pathogenic bacterial infection**.** Finally, the publication of the study findings in peer-reviewed journals and presentation in scientific meetings will be considered.

4. RESULTS

4.1 Socio-Demographic Characteristics

This study included 250 pediatric patients under the age of five who presented with diarrhea. The average age of the participants was 22 months, with a standard deviation of 3.85 months. Among them, 142 (56.8%) were male, and 108 (43.2%) were female. Regarding residence, 150 (60%) of the children were from urban areas, while 100 (40%) were from rural areas. In terms of the guardians' educational background, 90 (36%) could read and write, 89 (35.6%) were unable to read and write, and 20 (8%) had completed college or university education. Most guardians 97 (38.8%) were housewives, while 28 (12.2%) were civil servants.

Household characteristics revealed that 87 (34.8%) families had 2–3 members, 84 (33.6%) had 4–5 members, and 79 (31.6%) had more than five members. Additionally, 205 (82%) of households reported only one member with diarrhea, while 45 (18%) reported more than one affected member (Table 1).

Table 1: Socio-demographic characteristics of the study participants at Worabe Comprehensive Specialized Hospital, Central Ethiopia, 2024

Variables	Category	Frequency	Percentage%
Age in month	0-11	74	29.6
	12-59	176	70.4
Gender	male	142	56.8
	Female	108	43.2
Residences	Rural	100	40.0
	Urban	150	60.0
Educational background of the guardian	Unable to read and write	89	35.6
	Able to read and write	90	36.0
	Primary school	69	27.6
	College /university	2	8.0
Occupation of caregivers	civil servant	28	12.2
	Merchant	53	21.2
	Housewife	97	38.8

	Farmers	37	14.8
	Daily laborer	35	14.0
Family size	2-3	87	34.8
	4-5	84	33.6
	>5	79	31.6
No. of household members reported diarrhea	only one	205	82.0
	>one	45	18.0

4.2. Prevalence of Intestinal protozoa

The overall prevalence of intestinal protozoan parasitic infections among children under five with diarrhea was found to be 28.7% (72/250), with a 95% confidence interval (CI) of 25.9–37.8%. Two common intestinal protozoa identified were *G. lamblia* and *E. histolytica/dispar* giving a prevalence of 19.5% (49/250) and 9.3% (23/250), respectively (Figure 4).

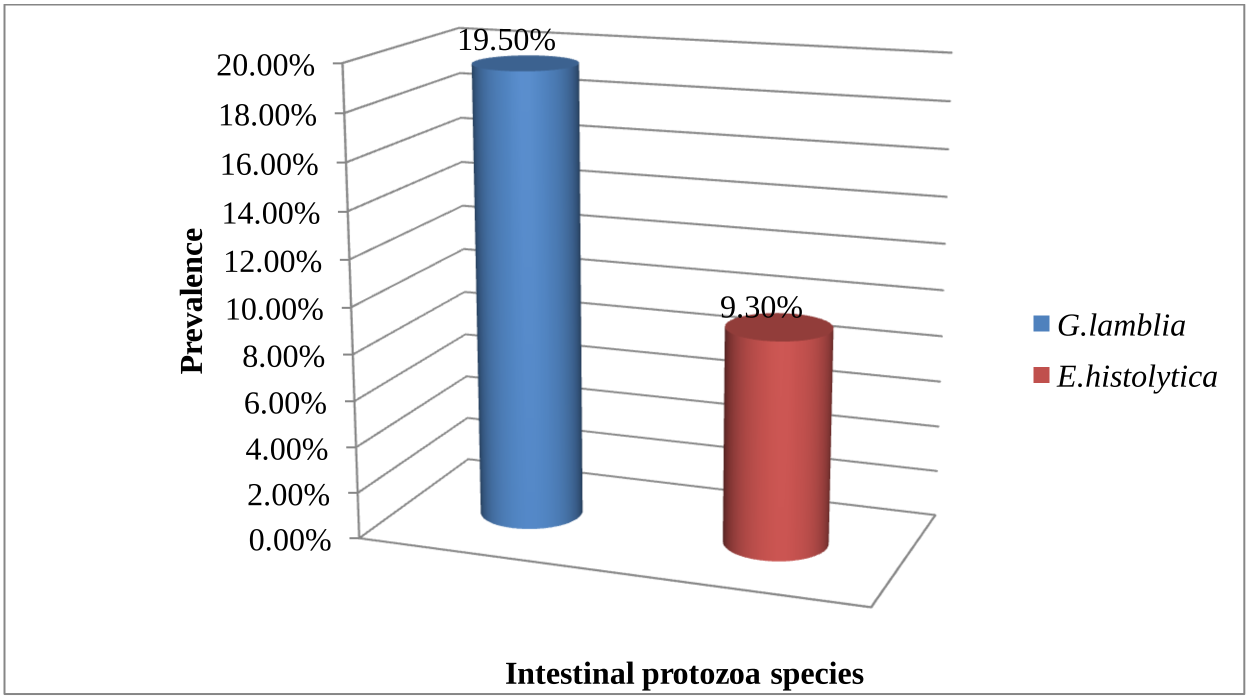

Figure 4: Prevalence of intestinal protozoa isolated from 250 diarrheic children.

4.3. Prevalence of Enteropathogenic Bacterial

The overall prevalence of enteric bacterial pathogens among under five diarrheic pediatrics patients in Worabe Comprehensive Specialized Hospital was (13.2%) (33/250) 95% CI (9.3-18). *Shigella spp.* 6.4% (16/250), and *Salmonella* spp. 6.8% (17/250) was the isolated pathogens from study participants (Figure 5).

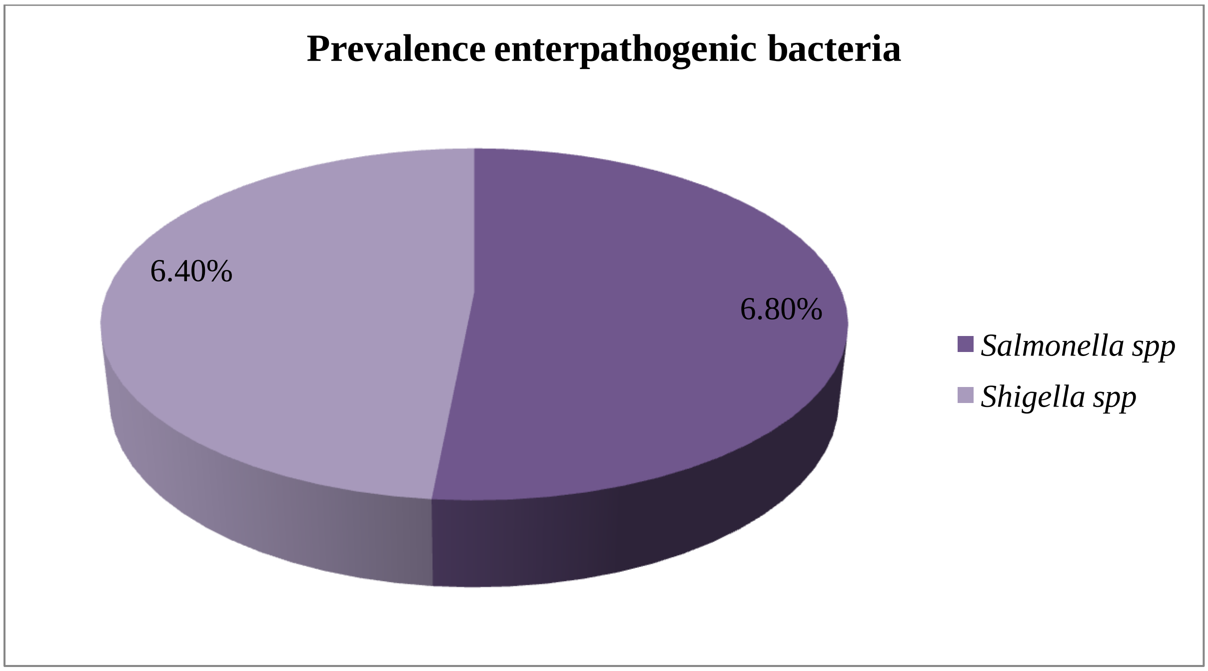

Figure 5: Prevalence of Enteric Bacteria isolated from 250 diarrheic children.

4.4. Prevalence of co-infection

In this study, there was a co-infection between protozoa (*E.histolytica/dispar* and *G.lamblia*), and bacteria (*Salmonella* and *Shigella species*) among under five diarrheic children. The prevalence of co-infection was 20/250 (8%) with (95% CI, 5% - 12.1%). The commonest co-infections were *G.lamblia and Salmonella spp. 7/250* (2.8%), *E.histolytica/dispar* and *Shigella spp. 6/250* (2.4%), *G. lamblia* and *Shigella spp.5/250* (2%), and *E.histolytica/dispar* and *Salmonella spp*. 2/250 (0.8%) (Figure 6).

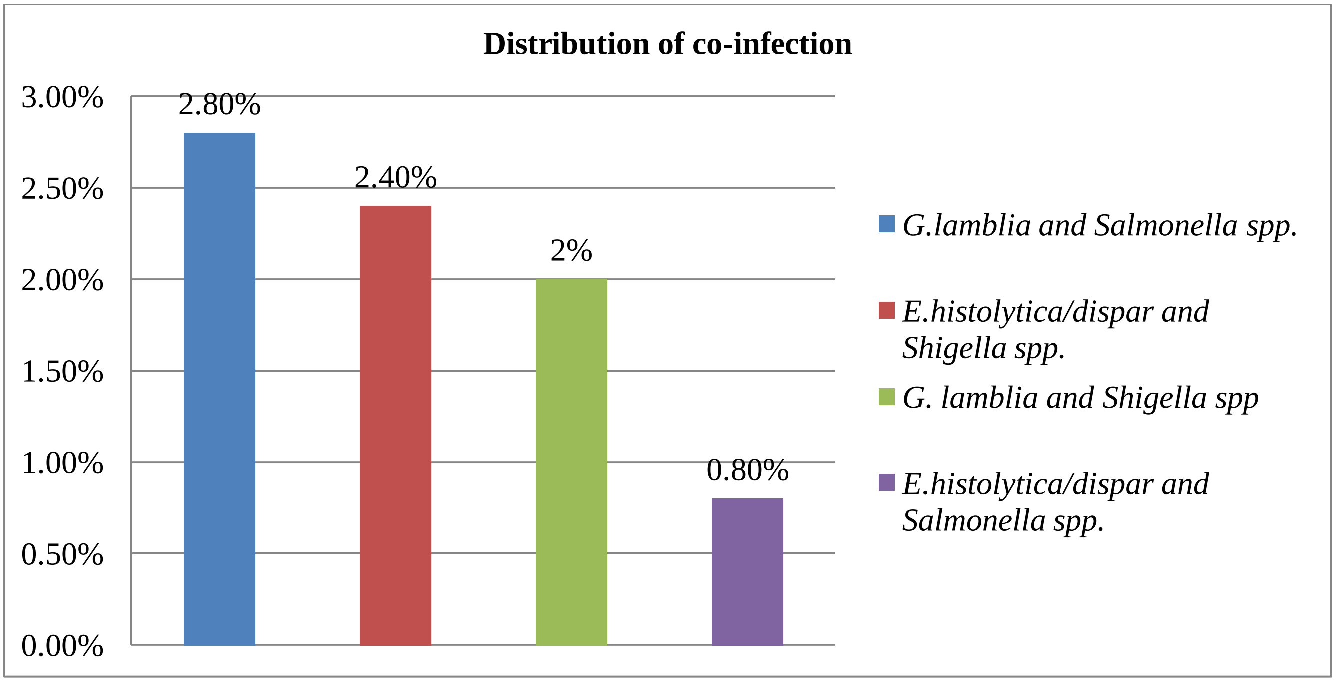

Figure 6: Prevalence of co-infection between enteric protozoa and bacteria isolated from 250 diarrheic children

4.5. The prevalence of other intestinal parasites

In this study, 7.2% (18/250) of the stool specimens had contain other intestinal parasites. *Ascaris lumbricoides* 3.6% (9/250), *Hymenolepis nana* 2.4% (6/250), and *Enterobius vermicularis* 1.2% (3/250) were the isolated intestinal parasites from study participants (Figure 7).

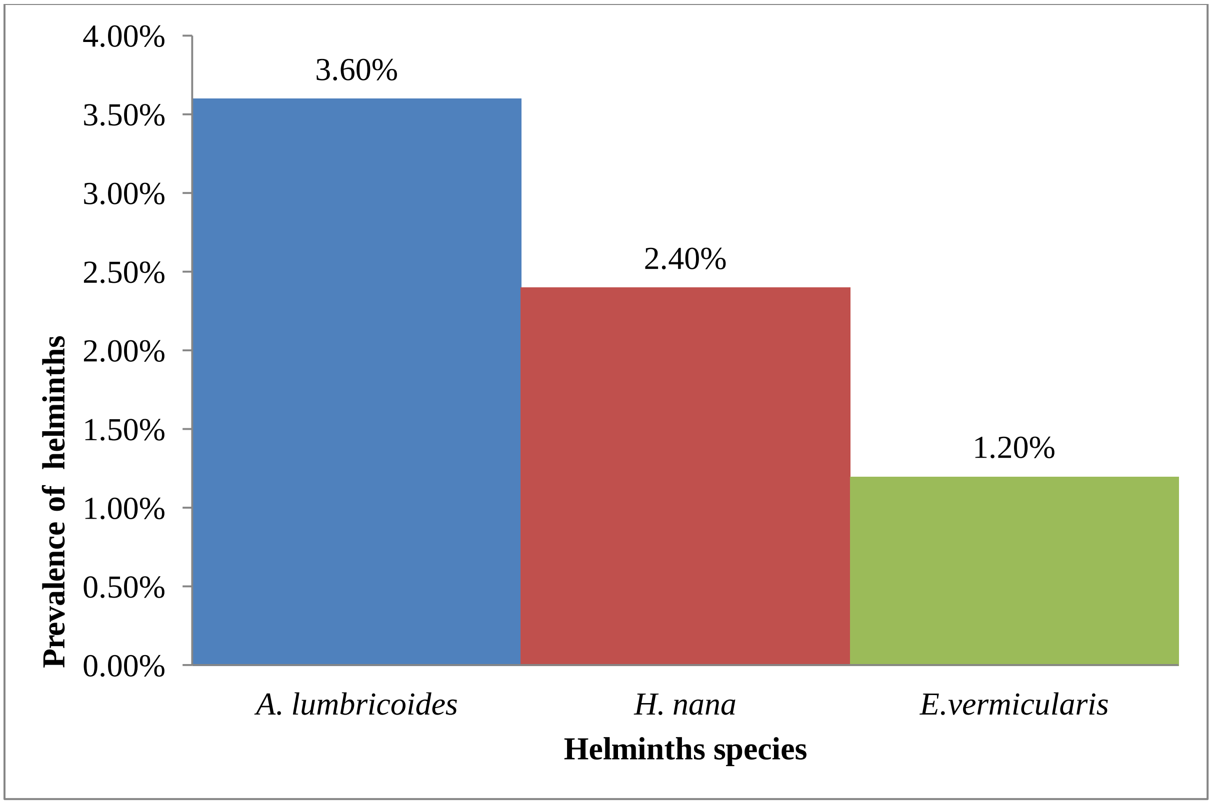

Figure 7: prevalence of other intestinal helminths isolated from 250 diarrheic children

4.6. Factors Associated with Intestinal Protozoa and EnteroPathogenic Bacteria

4.6.1. Bivariate analysis

Based on the bi-variable analysis, several factors were found to have a significant association with diarrhoeagenic enteropathogenic protozoa and bacteria, with a p-value of less than 0.25. These factors include the child's age, place of residence, family size, and hygiene practices such as washing children’s hands after defecation, the guardians' fingernail cleanliness, and the habit of guardians washing their hands before feeding. Other significant factors include washing vegetables or fruits before feeding children, consuming raw vegetables, the absence of a latrine, waste disposal practices, the presence of domestic animals in the household, the source of drinking water, and the type of diarrhea observed (Table 2).

Table 2: Bivariate analysis for factors associated with Intestinal Protozoa and pathogenic Bacteria

Risk factors	Response	Infected	Non Infected	Total	Bivariate result COR 95% CI	P. value
Age in month	0-11	3	71	74	1	
	12-59	17	159	176	2.53(0.719-8.9)	0.148
Residences	Rural	16	134	150	2.86(0.93-8.84)	0.067
	Urban	4	96	100	1	
Family size	<3	3	81	84	1	
	3-5	4	75	79	3.29(1.03-10.57)	0.018
	>5	13	74	87	4.74(1.3-17.3)	0.045
Washing pediatrics' hands after defecation	Daily	9	69	78	1	
	Sometimes	5	62	67	1.67(0.51-5.088)	0.411
	Never	6	99	105	2.15(0.7-6.32)	0.163
Fingernail Status of guardians	Trimmed	8	132	140	1	
	Untrimmed	12	98	110	3.43(1.28-9.3)	0.015
Hand washing before feeding the child	No	17	106	123	6.63(1.89-23.24)	0.003
	Yes	3	124	127	1	
Washing fruits/vegetables before eating	Daily	5	120	125	1	
	Sometimes	7	65	72	1.65(0.56-4.88)	0.364
	Never	8	45	53	4.27(1.33-13.73)	0.015
A habit of eating raw	No	6	119	125	1	

vegetables	yes	14	111	125	2.5(0.93-6.74)	0.07
Latrine availability	No	16	97	113	5.49(1.78-16.9)	0.003
	Yes	4	133	137	1	
Domestic animals in the house	No	3	117	120	1	
	yes	17	113	130	8.9(2.9-27.75)	0.006
Source of drinking water	Unprotected	16	105	121	4.76(1.54-14.68)	0.007
	Protected	4	125	129	1	
Waste disposal site	Hole	3	76	79	1	
	Municipal	3	52	55	2.34(0.65-8.65)	0.18
	Open field	14	102	116	3.5(0.97-12.57)	0.057
Type of diarrhea	Semi-fluid	10	39	49	1	
	Watery	4	44	48	2.56(0.65-10.14)	0.18
	Mucoid	3	31	34	2.65(0.67-10.45)	0.164
	Bloody	3	30	33	2.8(0.819-9.37)	0.100

COR= crude odd ratio, CI =confidence interval

4.6.2. Multi-variant analysis

The multivariate analysis conducted in this study revealed no significant differences between co-infection and several variables, including age, residence, hand washing habits after defecation among children, consumption of raw vegetables, waste disposal methods, and types of diarrhea. However, significant associations were found for other variables including, family size, fingernail status of guardians, hand washing habits of guardians before feeding children, washing fruits or vegetables before consumption, drinking water source, availability of a latrine, and presence of domestic animals in the household, all with a P-value less than 0.05 (see Table 8).

Specifically, the likelihood of intestinal protozoa and bacterial infection was statistically significant individuals from families with more than five members were found to be 11.59 times more likely to harbor enteric pathogens compared to those from families with fewer than three members (AOR = 11.59, 95% CI = 1.7 – 78.3).

Children with guardians who had untrimmed fingernails showed an 8.15-fold increase in the likelihood of enteric pathogen isolation compared to those whose guardians maintained trimmed fingernails (AOR = 8.15, 95% CI = 1.6 – 42.4). Additionally, children whose guardians did not wash their hands before feeding children were 6.63 times more likely to be infected with enteric pathogens than those whose guardians did (AOR = 6.63, 95% CI = 1.89 – 23.2). Notably, children who consumed unwashed vegetables and fruits were at a 25.4 times higher risk of having enteric pathogens compared to those whose produce was washed daily (AOR = 25.4, 95% CI = 2.96 – 217.3).

Using unprotected drinking water significantly increased the likelihood of enteric pathogens by 30.6 times compared to those using protected water sources (AOR = 30.6, 95% CI = 3.1 – 305.3). Moreover, the absence of a latrine was associated with a 23.4 times higher likelihood of pathogen compared to households with latrines (AOR = 23.4, 95% CI = 3.7 – 146.7). The presence of domestic animals in the household was also a risk factor, showing a 2.716 times increase in the likelihood of pathogen isolation compared to homes without such animals (AOR = 13.3, 95% CI = 1.6 – 110.9) (Table 3).

Table 3: Multivariate analysis of factors associated with Intestinal Protozoa and pathogenic Bacteria

Risk factors	Response	Infected	Non Infected	Total	multivariate result AOR 95% CI	P. value
Family size	<3	3	81	84	1	
	3-5	4	75	79	8.7 (1.08-70.06)	0.012*
	>5	13	74	87	11.59 (1.7-78.3)	0 .042*
Fingernail status of guardians	trimmed	8	132	140	1	
	Untrimmed	12	98	110	8.15 (1.6-42.4)	0.013*
Washing of vegetables or fruits before eating	daily	5	120	125	1	
	sometimes	7	65	72	1.65(0.56-4.88)	0.364
	never	8	45	53	25.4(2.9-217.3)	0 .003*
A habit of washing the guardian's hands before feeding children	No	17	106	123	6.63(1.89-23.240	0.020*
	Yes	3	124	127	1	
Do you have a latrine?	No	16	97	113	23.4 (3.7-146.7)	0.001*
	Yes	4	133	137	1	
Presence of Domestic animals in the house	No	3	117	120	1	
	yes	17	113	130	13.3 (1.6-110.9)	0.017*
Source of water for drinking	Unprotected	16	105	121	30.6(3.1-305.3)	0.004*
	Protected	4	125	129	1	

AOR = adjusted odd ratio, CI= confidence interval and *= indicates significant variables

5. DISCUSSION

Diarrhea continues to be a leading cause of morbidity and mortality among children under five, particularly in low-resource settings like Ethiopia. This study identified multiple enteric pathogens, including protozoa such as *G. lamblia* and *E. histolytica*, and bacteria like *Salmonella* and *Shigella spp.*, which contribute significantly to the burden of diarrheal diseases in pediatric populations.

The prevalence of intestinal protozoa, specifically *Giardia lamblia* and *Entamoeba histolytica*, was found to be 28.7% (95% CI: 25.9-37.8), indicating a notable public health concern, especially in comparison to other regional findings. This rate is higher than previous findings from Mozambique (21%) (9) and Bahir Dar town (20.4%) (39), suggesting local factors, including environmental, social, and health infrastructure conditions may contribute to increased transmission in this population. However, this rate remains lower than those reported in areas such as Yamoussoukro, Côte d'Ivoire (62.75%) (38); Eastern Tigrai (59.3%) (41); Zeita Primary School in Central Ethiopia (46.8%) (8); Atat Hospital in the Gurage zone (45%) (40); and Yirgalem Hospital, Ethiopia (49.56%) (70). These regional variations may reflect differences in sanitation, healthcare access, and local efforts toward parasite control typically involve community-based actions and public health programs, underscoring the need for context-specific interventions to reduce the burden of protozoan infections in this area.

In terms of bacterial pathogens, *Salmonella* and *Shigella* species are common bacterial pathogens causing acute diarrhea globally (71). In Ethiopia, where underdevelopment and tropical conditions prevail, *salmonellosis* and *shigellosis* are prevalent. This study identified the prevalence of *Salmonella* and *Shigella* among under-five pediatric patients to be 13.2% (95% CI: 9.3-18). This finding aligns with earlier studies from the Central African Republic (10.84%) (44) and Addis Ababa (10%) (45), indicating that the burden of bacterial diarrheal diseases in Ethiopia aligns with other regions facing comparable socio-economic and environmental conditions. However, this prevalence is notably higher than reported from Altamura Health Center (8%) (46), Ambo town (3.8%) (48), and Hossana (9.3%). However, it is lower than studies from Dera Ismail Khan (25%) (53) and Debre Berhan (46%) (47). This variation in bacterial prevalence underscores the influence of factors such as community hygiene practices, healthcare infrastructure, and possibly climates such as temperature, humidity, and precipitation are affect the survival and transmission of bacterial pathogens

Most research on acute diarrhea in children has primarily focused on single-pathogen infections. This study is one of the few laboratory-based investigations that examined co-infections between enteric protozoa and bacteria in under five children with diarrhea. The presence of co-infections, particularly combinations of bacterial and protozoan pathogens, may have important implications for treatment protocols, especially in developing countries where empirical treatments are common. The simultaneous detection of multiple pathogens has been linked to increased severity of clinical symptoms compared to mono-infections.

The prevalence of co-infection in this study was 8% (95% CI: 5% - 12.1%), which is lower than the rates observed in South Africa (12.5%) (72) and East Gabon (25.5%)(73). Several factors may explain these differences, such as variations in study design, population characteristics, diagnostic methods, and the local epidemiological context.

The co-occurrence of *Shigella* and *Giardia lamblia* was observed at 2%, consistent with earlier studies. A study in South Africa reported a similar prevalence of 2.1% (72), while a study in East-Central Gabon found a rate of 1.9% (73). This consistency across regions suggests a stable co-infection between these pathogens in Africa, possibly due to shared environmental or socio-economic factors that influence transmission.

The co-infection for *Salmonella* and *Giardia lamblia* was 2.8% (95% CI: 2.1% - 4.6%), lower than the 4.8% reported in previous studies from Addis Ababa (49). This difference may reflect variations in environmental conditions or public health measures that affect pathogen exposure to risk factors in different communities. Additionally, the co-infection for *Salmonella* and *E. histolytica* was 0.8% (95% CI: 0.12% - 3.6%), significantly lower than the 9.7% prevalence reported in Addis Ababa (49). This discrepancy could be due to factors such as dietary habits, particularly the consumption of raw foods, which may influence pathogen transmission.

The factors contributing to co-infection in pediatric patients are complex and vary according to socioeconomic, environmental, and behavioral contexts (47). This study identified several associated factors. A larger family size was associated with a higher likelihood of infection compared to smaller families, aligning with findings from Gondar, Ethiopia (74). This may be due to limited resources and inadequate care for children in larger families. Additionally, children whose guardians had untrimmed fingernails were at a greater risk for infections, supporting findings from Arba Minch, Ethiopia (73), which suggest that untrimmed nails can harbor protozoan cysts and bacteria.

This study revealed that conditions surrounding drinking water sources also showed significant variation in the prevalence of infections. This aligns with previous findings from Bahir Dar, Ethiopia (32), suggesting a higher risk of pathogen exposure due to contaminated water sources. Guardians who did not wash their hands before feeding children were more likely to facilitate infections compared to those who practiced hand hygiene. This finding is consistent with earlier studies in several regions of Ethiopia (32,34,50,72), indicating that poor hand-washing practices increase the risk of diarrheal diseases.

The presence of domestic animals in the household correlated with a higher likelihood of diarrhoeagenic protozoa and bacteria, contrasting with findings from Ambo town, Ethiopia (48) The discrepancy between the two studies may be attributed to differences in geographic, socioeconomic, and cultural factors that influence animal management practices, environmental contamination, and exposure risks. Additionally, not washing vegetables or fruits before feeding children was associated with higher infection rates, which reflects differences in cleanliness practices and the potential for soil-transmitted pathogens.

The absence of latrines in residential areas significantly contributed to acquiring both protozoan and bacterial infections among children. This study found a strong association between the lack of latrines and the presence of intestinal pathogens, mirroring a study conducted in Eastern Ethiopia (59). This underscores the implications of poor sanitation, where inadequate waste management can lead to increased pathogen transmission through contaminated water.

6. LIMITATIONS

This study faced several challenges due to limited resources. Isolation of *Salmonella* and *Shigella* strains was not conducted. Similarly, the absence of specialized equipment, such as microaerophilic environments and selective media, prevented the isolation of key enteropathogenic bacteria like *EHEC O157:H7*, *Campylobacter species*, and *Clostridium difficile*, which are common causes of diarrhea.

Additionally, the study had a few inherent limitations:

Sampling Bias: Inconsistencies during sampling in the parent study resulted in some samples being smaller than the required volume, potentially affecting the results.

Identification Challenges: Using light microscopy, it was not possible to distinguish between *Entamoeba histolytica* and *Entamoeba dispar*. While both were considered diarrhea-causing parasites for this study, only *E. histolytica* is pathogenic, and *E. dispar* is typically more prevalent.

7. CONCLUSION AND RECOMMENDATIONS

7.1. Conclusion

This study highlights a significant rate of co-infection of enteric protozoa and bacteria among children under five suffering from diarrhea at Worabe Hospital in Central Ethiopia. The findings point to several contributing factors, such as the untrimmed fingernails of caregivers, the presence of domestic animals in the household, caregivers' lack of handwashing habits before feeding children, large family sizes, the absence of latrines, and the failure to wash vegetables and fruits before feeding children. Addressing these factors is critical to reducing diarrhea cases and enhancing the overall health of children in the region.

7.2. Recommendation

Enhance Sanitation and Hygiene: Implement community-based programs that promote proper sanitation and hygiene practices, including hand washing education and access to clean water, to reduce the transmission of pathogens.

Routine Screening and Diagnosis: Establish routine screening protocols for enteric infections in pediatric care settings, enabling early diagnosis and treatment of co-infections to prevent severe outcomes.

Further Research: Encourage further studies to explore the long-term effects of co-infections on child health and the socio-economic factors influencing these infections, enabling data-driven public health strategies.

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