KIBOGORA POLYTECHNIC

FACULTY OF HEALTH SCIENCES

DEPARTMENT OF BIOMEDICAL LABORATORY SCIENCES

THE INCIDENCE OF AMOEBIASIS AND ASSOCIATED RISK FACTORS IN PREGNANT WOMEN ATTENDING MUSHUBATI HEALTH CENTER DURING 2022 - 2023

Case study: Mushubati Health Center, Rutsiro District

Period: January, 2022- July, 2023

Undergraduate research thesis submitted in partial fulfilment of the requirements for the Bachelor's degree with honor in Biomedical Laboratory Sciences.

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August, 2023

DECLARATION

Declaration by the Candidates

We IHIMBAZWE Gentille and TWAMBAZEMARIYA Clemence hereby declare that this is our own original work and not a duplication of any similar academic work. It has therefore not been previously or concurrently submitted for any other degree, diploma or other qualification to Kibogora Polytechnic or any other institution. All materials cited in this paper which are not my own have been duly acknowledged.

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Signed.....

Date.....

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Declaration by the Supervisor

I declare that this work has been submitted for examination with my approval as KP Supervisor

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Date…….

ABSTRACT

Amoeba infections during pregnancy have been linked to an increased risk of complications and negative pregnancy outcomes like maternal anemia, miscarriage, intrauterine growth retardation, and maternal death. (KALILAN L, 2020) .the aim of this study was (1) To determine the prevalence of amoebiasis among pregnant patients at Mushubati Health Center. (2) To assess risk factors associated with amobiasis among pregnant patients at Mushubati Health Center. (3) To determine water utilized among pregnant patients at Mushubati Health Center. In order to determine the prevalence of amoebiasis among pregnant women who visited Mushubati Health Center in 2022 to 2023, this study employed a retrospective design and a quantitative approach, emphasizing objective measurements and statistical analysis of data obtained through a data collection sheet. The study made use of statistical measurement (numbers) or data analysis using numbers. Amoebiasis was discovered in 31.81% (n=35) of pregnant women. The study shows that 31.81% (n=35) of participants had amoebiasis, with 20% (n=22) of these being negative, and 50% (48.18% (n=53) having other parasites. For the risk associated with amoebiasis study showed that washing food before cooking accounted for 54% of factors associated with amoebiasis among pregnant women, cleaning kitchen materials with pipe water and detergent accounted for 36%, kitchen always cleaned accounted for 48%, and hand washing after toilet accounted for 40%. For water utilization study showed that many participants use stagnant water at 56.36% pipe water at 45.45%.Washing food before cooking Cleaning kitchen materials Hand Washing after toilet was statically significantly where P-value was <0.05. Usage of Stagnant water (P =.0000) was statically significantly for causing amoebiasis. The study area had a high prevalence of amoebiasis, which still posed a health risk to pregnant women. To lessen the burden of infections among pregnant women, we advise strengthening sanitation and hygiene programs as well as routine deworming of mothers-to-be.

DEDICATION

To:

The almighty God,

Our beloved parents,

All our brothers and sisters,

Our friends,

This study work is dedicated

ACKNOWLEDGEMENT

We would like to thank Almighty God who made all this possible. Our gratitude also goes to the Kibogora Polytechnic for giving us an opportunity to study at the University. we also grateful to department members of the Biomedical Laboratory Sciences for their academic support.

We express our heartfelt gratitude for our supervisor Mr. HITAYEZU Elysee for his valuable guidance, support, and mentorship throughout the process of completing our research thesis titled. His unwavering dedication, insightful feedback, and encouragement have been instrumental in shaping this project and guiding it towards successful completion.

We wish to convey our appreciations to our families for their understanding, patience and encouragement and last but not least, we are greatly indebted to our classmates and friends who have supported us during our study.

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LIST ABBREVIATIONS AND ACRONYMS

ANC: Antenatal Care

C I: Confidence Interval

CIE: Counter-Immunoelectrophoresis

DNA: Deoxyribonucleic Acid

ELISA: Enzymes Linked Immunosorbent Assay

GBD: Global Burden of Disease

GIS: Geographic Information Systems

IFA: Immunofluorescent assay

IgG: Immunoglobulin G

IHA: Indirect Hemagglutination

M.O.H: Ministry of Health

MDGs: Millennium Development Goals

NTDs: Neglected Tropical Diseases

PCR: polymerase chain reaction

RBCs: Red Blood Cells

SPSS: Stastical package for social sciences

CHAPTER ONE: GENERAL INTRODUCTION

1.0 INTRODUCTION

Amoebiasis poses significant community health concern especially in pregnant woman due to their physiological status. Pregnancy-induced immunodeficiency can lead to reduced IgA activities which makes pregnant women susceptible to penetration of intestinal mucosal linings and placental barrier. This chapter is constituted with the background of the study, statement of the problem, objectives of the study, research questions, significance of the study, and the scope of the study.

1.1 BACKGROUND OF THE STUDY

Amoebic infections are a serious health issue everywhere, but they are especially prevalent in tropical regions and areas with poor sanitation and public hygiene. After malaria and schistosomiasis, it is the third most common cause of parasitic infection-related mortality worldwide (park, 2017)

Amoebiasis is responsible for the deaths of about 0.1 billion people worldwide. Amoebiasis is a condition that affects immigrants, travelers, and people with immune deficiencies, primarily pregnant women (Hailu T, 2020)Low immunity during pregnancy makes it easier for infections from bacteria and parasites to spread (Bless Mann ., 2022). The disease is spread through the consumption of contaminated water or food. E. histolytica is responsible for many cases of dysentery and severe abdominal pain. The fecal-oral route is the most common route of E. histolytica infection by cysts transmission, and some reports have also found it in homosexual men ((khanam, 2019)

According to GBD (2015), amoebiasis is the fourth most common parasite infection that results (park, 2017)in death globally. Around 50 million patients experience amoebic dysentery or extra intestinal disease symptoms each year, and this unique pathogen is thought to be the cause of 50,000 to 100,000 annual deaths ((Shirley DT, 2019)

According to a study, amoebiasis affects 48% of pregnant women in Mexico, compared to infection rates of 25.4% in India, 31.9% in Africa, and 33.5% in Central and South America. According to Billet et al. (2019), amoebiasis is the third deadliest infection in the world. There is a 42% mortality rate with increasing age and late diagnosis, with the exception of pregnant patients

with severe amoebiasis. Pregnant women are the subject of more extensive research on amoebiasis ((YANAGAWA Y.ARISAKA T, 2019).

Several research investigations on the prevalence of amoebiasis among pregnant women have been conducted in Ethiopia. For example, the overall prevalence of amoebiasis among pregnant women attending prenatal care at Felege Hiwot Referral Hospital in Bahir Dar was 31.5% (Derso et al., 2016); 43.8% among pregnant women attending ANC at public health facilities in the Lalo Kile district, Oromia, Western Ethiopia (Yesuf D.A, 2019), 37.3% among pregnant women in West Gojjam Zone, Northwest Ethiopia (Hailu T, 2020)

Many factors, including low socioeconomic status, inadequate sanitation and personal hygiene, and a lack of clean water, affect the distribution of amoebiasis (Sapkota & Maharjan, 2027). Being a farmer, going barefoot, not washing your hands properly after using the restroom, living in a rural area, eating raw vegetables, using toilets improperly, having poor environmental sanitation, eating from the ground, using irrigation, and not receiving adequate health education were risk factors for pregnant women (Yesuf D.A, 2019)

1.2 STATEMENT OF THE PROBLEM

Due to lowered immunity, parasitic infections are more common in pregnant women and can therefore impact the body's physiological systems. According to (KALILAN L, 2020)amoeba infections during pregnancy have been linked to an increased risk of complications and negative pregnancy outcomes like maternal anemia, miscarriage, intrauterine growth retardation, and maternal death (KALILAN L, 2020)According to a study conducted in Kenya by Wekesa and colleagues, Amoebiasis infestations during pregnancy are linked to a higher risk of maternal complications and unfavorable perinatal outcomes, including anemia, low birth weight, and perinatal mortality. The risks of low birth weight, preterm delivery, stillbirth, fetal anemia, and fetal mortality may also be increased by parasitic infection (AW WEKESA, 2014) A systematic review found that amoebiasis infected 37.7 million women in Sub-Saharan Africa in 2015. Amoebiasis is the second leading cause of death among parasitic diseases after malaria, and 6.9 million of these were pregnant women (KAYASTHA, 2018)

According to research by (K RADON, 2018) pregnancy is a known independent risk factor for intestinal parasite infections, including amoebiasis. Knowing the prevalence of amoebiasis among pregnant women is important so that clinicians are aware of how they present, what is necessary

to make a diagnosis, and how to manage pregnant women with these infections. This is because parasitic infections can have serious effects on pregnancy, such as anemia, low weight gain, impaired fetal growth, preterm birth, and maternal mortality (Feleke , 2018).

There is a lacking of specific data on the prevalence, distribution, and disease burden of amoebiasis in Sub-Saharan Africa, despite the fact that limited sero-prevalence studies among pregnant women in SS Africa suggest that it may be common (Kamath, 2019). This study is being done to determine the prevalence of amoebiasis among pregnant patients at Mushubati Health center

1.3 PURPOSE OF THE STUDY

1.3.1 General objective

The aim of this study is to determine the prevalence of amoebiasis and associates risk factors in pregnant women attending Mushubati Health Center during 2022-2023.

1.3.2 Specific objective

1. To determine the prevalence of amobiasis among pregnant patients at Mushubati Health Center during 2022-2023(According to the age group, pregnancy trimester, parity)

2. To assess risk factors associated with amobiasis among pregnant patients at Mushubati Health during 2022-2023.

3. To determine water utilized among pregnant patients at Mushubati Health Center during 2022-2023.

1.4 Research questions

1. What is the prevalence of amobiasis among pregnant patients at Mushubati Health Center during 2022-2023?

2. What are risk factors associated with amobiasis among pregnant patients at Mushubati Health during 2022-2023?

3. What is water utilized among pregnant patients at Mushubati Health Center during 2022-2023?

1.5 SIGNIFICANCE OF THE STUDY

1.5 .1 Community interest

In order to help the health sector, develop strategies for controlling parasitic infection and lower maternal mortality and complications, this study will ascertain the prevalence of amoebiasis among pregnant women.

1.5.2 Mushubati heath center interest

Mushubati Health Center will gain from the research by creating defenses against amoebiasis infections in expectant women. Also the findings from this study will used by decision makers on elaboration of new protocols on treatment, prevention and control of this infection.

1.5.3 Personal interest

This study helps the researchers to improve knowledge and skills acquired in biomedical laboratory sciences, this research study will also allow the researchers to be rewarded Bachelor's degree with honor in health sciences

1.5.4 Scientific and academic Interest

This study will also benefit for academician as it may add new knowledge on existing literature and may serve as reference to other researchers who may be interested in the same area

1.6 LIMITATION OF THE STUDY

The study was limited by financial constraints, transportation costs, and the materials required to complete the study, as well as missing complete data.

1.7 SCOPE OF THE STUDY

1.7.1 Content Scope

Incidence of amoebiasis and associated risk factors in pregnant women attending Mushubati Health Center during 2022-2023was assessed.

1.7.2 Time scope

The study was carried out at Mushubati HC during 2022-2023

1.7.3 Geographical scope

The study was carried out at Mushubati Health Center, in Rutsiro District, Western province of Rwanda during 2022-2023.

CHAPTER TWO: LITERATURE REVIEW

2.0 INTRODUCTION

This chapter consists of the overview of relevant literature on amoeba and it is presented in the following manner: definition of key term, literature related to the objectives, Conceptual framework and Summary.

2.1 DEFINITION TERMS / KEY CONCEPTS

Prevalence: Statistical concept referring to the number of cases of a disease, which are present in a particular population at a given time

Infection: A condition marked by subjective complaints, a specific history, and clinical signs, symptoms, and laboratory or radiographic findings caused by microorganisms

Amoebiasis: is an infection of the intestines caused by a parasitic amoeba Entamoeba histolytica.

Pregnant: is containing a developing embryo, fetus, or unborn offspring within the body.

2.2 THE PREVALENCE OF AMOEBIASIS AMONG PREGNANT WOMEN

In developed countries, the infection occurs primarily among travelers, recent immigrants from endemic regions, and immuno-compromised persons (A AYADI, 2012)). The prevalence of E.histolytica among pregnant women was reported as 4% in the USA (park, 2017)Entamoeba histolytica is a major cause of morbidity worldwide, causing approximately 50 million cases of dysentery and 100,000 deaths annually (ESPINOSA A.F, 2018)Entamoeba histolytica associated dysentery is common in the less developed and developing countries of the world but is more common in areas of low socio-economic status, poor sanitation and nutrition, especially in the tropics (ESPINOSA A.F, 2018)

Majority of E. histolytica infections, morbidity and mortality among pregnant women occur in Africa, Central and South America and the Indian Sub-continent, where the prevalence of Entamoeba infection is as high as 50% (park, 2017)Entamoeba histolytica seroprevalence studies in Mexico revealed that more than 8% of pregnant women studied had this parasite (park, 2017). In endemic areas, as many as 25% of patients may be carrying antibodies to E. histolytica as a result of prior infections, which may be largely asymptomatic. The prevalence of asymptomatic E. histolytica infections seem to be region-dependent; in Brazil, for example, it may be as high as

11%. In Egypt, 38% of individuals presenting with acute diarrhea to an outpatient clinic were found to have amebic colitis (Park et al., 2017). In Hue City, Vietnam, the annual incidence of amoebic liver abscess was reported to be 21 cases per 100,000 inhabitants (Park et al., 2017). An epidemiologic study in Mexico City reported that 9% of the population was infected with E. histolytica in the 5-year to 10-year period preceding the study. A prevalence of 39% was recorded in Bangladesh and 33% in Columbia among pregnant women (Park et al., 2017).

In some African countries, 6% to 66% of the pregnant women had the parasite (Stark et al., 2016). These studies were conducted using microscopic examination, and therefore only indicate distribution of the disease. Such results require confirmation by techniques that clearly differentiate E.histolytica from E. dispar, which is not pathogenic. Other studies in parts of Africa reported prevalence rates of 22% and 21% in South Africa and Egypt respectively (Stauffer et al., 2016). In Nigeria, prevalence rates were 22.3% in Calabar (Ozumba, 2021), 21.6% in Enugu and 13.7% in Ilesa (Ogunlesietal, 2015). In Uganda the prevalence was 1.4% (Brink et al., 2022), Ethiopia was 10.3% (Hailemariam et al.,2022), and Dakar, Senegal was 5.1% (Gassama et al., 2011) and South Africa was 12.4% (Samie et al., 2016). Cases of the disease are reported in different health facilities in Kenya, for example, in Njoro PCEA health center, Nakuru County, prevalence rate of 21% was reported (Kinuthia, 2022**).**

Symptomatic intestinal amoebiasis occurs in all age groups of pregnant women. Liver abscesses due to amoebiasis are 10 times more frequent in adults than young pregnant mothers. Very young pregnant mothers seem to be predisposed to fulminant colitis (Davies, 2016). Amoebic dysentery is very rare in young in pregnant women while amoebic colitis is common in adult to middle-aged adult pregnant women of between 25 years to 40 years (WHO, 2021).

Amoebic colitis affects all age of pregnant women equally (Davies, 2016). However, invasive amoebiasis is much more common in young old pregnant mothers than young pregnant mothers. In particular, amebic liver abscess is 7-12 times more common in old pregnant mothers than in young pregnant mothers, with predominance among women aged 18-50 years. The reason for this disparity is unknown, though hormonal effects are implicated, as the prevalence of amebic liver abscess is also increased among postmenopausal women (khanam, 2019)Among pubertal amebic liver abscess is equally common in both ages groups among pregnant women (Davies, 2016). Acuna-Soto and co-authors, 2022, noted that asymptomatic E. histolytica infection is distributed

equally between all age group of pregnant women and that higher proportion with invasive amoebiasis may be due to a female higher susceptibility to invasive disease (Acuna-Soto , 2022).

Regarding amoebiasis study conducted Sao Tome and Principe found a 3% prevalence of Entamoeba histolytica in the women with prima while the gravida 2 had a 4.2% of amoebiasis (Gomes et al., 2022). Overall estimates report that 10 million women in Africa per year have amoebiasis during pregnancy. This paucity of data can be related to the fact that Entamoeba histolytica is restricted to a few central African countries, is transmitted by poor hygiene and poor sanitation they also observed that most pregnant women from our study with infection had an urban residence and also they found that pregnant women with more 2 para had high rate of infection than other categories (Wekes , 2017).

Several studies demonstrated very high prevalence of amoebiasis among pregnant women with more two parities than other categories in different parts of Ethiopia , However, there are still localities in the country, including the present study area, Alefa District, for which information was lacking ((DERSOA, 2016)

2.3 RISK FACTORS ASSOCIATED WITH AMOEBIASIS

Environmental, socio-economic, demographic, low education level and hygiene related behavior influence the transmission and distribution of intestinal amoebiasis infections (Norhayati et al., 2023). A study done in Brazil associated place of residence, age, intake of raw / poorly cooked vegetables and quality of drinking water as major risk factors. Prevalence of amoebiasis is more related to poor environmental sanitation and personal hygiene than to climate. Socio-economic factors as well as unpredictable factors such as food insecurity, droughts, and floods contribute to the problem (WHO, 2021). Unavailability of safe domestic water and low education on sanitation also contribute to transmission (AMREF, 2019).

Most amoeba parasites gain entry into the intestines through the mouth from undercooked food, vegetables, or contaminated water or hands, hookworm larvae penetrate skin. Poor personal hygiene, poor garbage disposal and poor disposal of human excreta are significant for this oral-faecal infection. Infective eggs may contaminate vegetables when untreated human stool of infected individuals is used as fertilizer for food crops. Infection may also take place when food is

handled without killing or removing the infective eggs on hands, clothes, hair, raw vegetables / fruit, or cooked food that is (re)infected by handlers, containers, etc (Blackwell , 2015). Poor hygiene, lack of access to drinkable water, poverty, illiteracy, and a hot, humid tropical climate are the main causes of it. Therefore, the objective of the current study was to evaluate the prevalence rate and associated factors of E. histolytica. (MOHAMMED KUDDUS, 2022)E. histolytica from E. dispar and E. moshkovskii, which are not pathogenic. As shown by this study, contaminated veggies and poor hygiene practices are more likely to blame for the dynamics of transmission. This study also demonstrated that direct human touch can spread disease. However, domestic animals' contribution to the spread of the Entamoeba complex (Al-Mekhlafi, 2012)

2.4 ASSOCIATION OF WATER UTILIZED

Since amoebiasis can be transmitted through the oral-fecal route and contaminated water, the prevalence of amoebiasis infections countries that are facing the lack in safe drinking water and suitable sanitation facilities like low and middle-income countries and rural areas is relatively high (DERSOA, 2016)Based on the reports, about 2.5 million and 780 million people have no access to sanitation facilities and safe drinking water, respectively. Additionally, considerable geographical heterogeneity in the safe drinking water supply and sanitation facilities was observed in developing countries (DERSOA, 2016)

Since several contradictory studies have been conducted on the risk of intestinal *Entamoeba spp* infection in the communities with the lack of safe drinking water and sanitation facilities (Hailu T, 2020)), a systematic review and meta-analysis can help us to elucidate the association of contaminated drinking water consumption and live in poor sanitation with the risk of intestinal *Entamoeba spp* infection ((DERSOA, 2016)

Sanitation plays a significant role in transmission and prevalence, in adequate sanitation can contribute to the spread of amoeba, E. histolytica same specific ways I which poor sanitation acts as a risk of amoebiasis: contaminated water sources, lack of clean water improper waste disposal, poor hygiene facilities (cm ajero, 2008). Lack of access to clean water, in region without access to

clean water source individual may have no choice to use contaminated for various purposes including drinking, cooking and personal hygiene. This perpetuate the cycle of amoebiasis transmission. Poor water treatment and storage, in areas with inadequate water treatment systems or where water storage practices are unsanitary, the risk of waterborne diseases like amoebiasis escalates (priti karadhajne, 2020).

Amoebiasis, caused by *Entamoeba histolytica*, has a worldwide distribution and is of public health significance in many developing countries. It has a fecal–oral transmission cycle and is most prevalent in developing countries in regions where substandard sanitary conditions exist due to poverty. Little is known about the epidemiology of *E. histolytica* infection and its presence in different socioeconomic communities in developing countries. We undertook the present study in the city of Lahore, Pakistan, and our prediction was that the prevalence of *E. histolytica* (Maqbool, et al., 2015)

2.5.1 Classification of Entamoeba histolytica

Description of Entamoeba species depend on the features of the parasites, such as the size of the trophozoites and cysts, the number of nuclei in the mature cyst and the number of structures in the trophozoites ((ABD-ALLA, 2019)E. histolytica is the only pathogenic species and its classification has been proposed (ABD-ALLA, 2019)as shown below: Kingdom Animalia Phylum Sarcomastigophora Sub-phylum Sarcodina Class Lobosea Family Entamoebidae Genus Entamoeba

2.5.2 Morphology

Trophozoites

The trophozoite of E.histolytica vary in size from 15-60μm, its shape is not fixed because of constantly changing position (Ichhpujani and Bhatia, 2013). They move by pseudopodia which are cytoplasmic protrusions that may be formed at any point on the surface of the organism. Motility is usually progressive and directional; its cytoplasm is divisible in two portions: a clear translucent ectoplasm and a granular endoplasm (Ichhpujani and Bhatia, 2013). Red blood cells, white blood cells and tissue debris may be occasionally seen inside the cytoplasm (Ichhpujani and Bhatia, 2013). There is only one nucleus which is 4-6μm in size, spherical in shape and placed eccentrically (Kenneth et al, 2020). The nucleus has a clear membrane, the inner surface of which

is lined with uniform and closely packed fine granules of chromatin; karyosome consists of several granules (Kenneth et al, 2020).

Cyst

During encystement the parasite is surrounded by a smooth, refractile non-staining wall which is about 0,5µm in thick. The size of the cyst varies between 12-15µm, the nucleus retains the characters of the trophozoite (Burton , 2015). Early in the development the cytoplasm of the cyst shows 1-4 chromatoid bars which are refractile, rounded end, the cyst is initially uninuclear but by binary fission soon develops into a binuclear and quadrinuclear body, as cyst matures both the glycogen mass and the chromadal bars generally disappear (Burton , 2015).

2.5.3 Transmission of Entamoeba histolytica

Transmission occurs by one of several ways, via ingestion of fecally contaminated food or water containing cysts or by oral-anal sexual contact (Engelkirk , 2020).

Human beings are the primary known reservoir for E. histolytica (Katz ., 2017). The main source of transmission is the chronically infected human who pass stools infected with cysts of E. histolytica that may contaminate fresh food or water through - 25 - poor personal hygiene (Ravdin, 2002). Another common source of transmission is oral-anal sexual contact (Quinn ., 2016). Zoonotic transmission of E. histolytica has been suggested by Jackson ,2020), but this is not clear. Experimental infections with E. histolytica have been produced in dogs, cats, rats, monkeys and other laboratory animals. These animals may acquire human strains as a result of close contact with humans. Natural infections with strains morphologically similar to E. histolytica have been found in monkeys (Beaver., 2015). The importance of wildlife (primates) in zoonotic infections was studied by Jackson 2020), who used zymodeme analysis to investigate whether E. histolytica occurs as a true zoonosis. In Nigeria, Mbaya and Nwosu (2015) reported clinical amoebiasis among captive chimpanzees at the Sandal kyarimi park, Maiduguri. They concluded that the animals must have acquired E. histolytica infection from one of the human attendants that was shedding the cysts in his faeces at the time of the outbreak. According to Walsh (2020), the infective cysts of E. histolytica may be spread by arthropods such as cockroaches and flies, suggesting that these insects are able to play a rare but in transmission of this disease

2.5.4 Pathogenicity

Following the ingestion of cysts and excystation in the small bowel, trophozoites colonize the colon. If trophozoites are abundant, there is an increased chance that some will make contact with mucosa long enough to grow, multiply and eventually invade the tissues (priti karadhajne, 2020) Several factors contribute to the pathogenicity of E.histolytica. Three such factors: Adherence of trophozoites to host cells predominantly mediated by a galactose/N-acetylgalactosamine

inhibitable surface lectine (Sidney, 2018). Killing of host cells by pore-forming peptides known as amoebapores. Proteolysis of the host's extracellular matrix mediated by cysteine proteinases (priti karadhajne, 2020)

This surface lectine mediates attachment of E. histolytica trophozoites to human colonic mucosa and submucosa (Sidney, 2018). In addition, the lectin has been implicated in cytolytic process; another interesting feature of the lectin is that it binds to purified C_8 and C_9 complement components, blocking the formation of the complement membrane attack complex on the amoebic plasma membrane. This suggests a possible role in mediating amoebic resistance to complement lysis through components C_8 and C_9 (Braga , 2012).

Amoebapores are small group of pore-forming protein contain in cytoplasmic granules of the trophozoites. They are able to bind and insert into lipid membrane forming ion channels, changing the content of the target and ultimately resulting in its lysis (Leippe and herbust, 2014). There are three amoebapores forms: A, B and C (Leippe and herbust, 2014). Although C is least abundant, it has substantially higher cytolytic efficacy (Leippe and Herbst 2014). Amoebapore C is virtually

abscent from Entamoeba dispar, possibly explaining its inability to destroy nucleated cells (Leippe and Herbst 2014). Cysteine proteinase is considered to be significant virulence in the pathogenesis of amoebiasis and have suggested playing vital roles in tissue invasion, and modulation of the cell-mediated immune response (Reed , 2020). During their passage to deeper layers of intestine, trophozoites must lyse extracellular matrix components of the colonic mucosa (Ortega, 2016).

Bhatia, 2003). There is only one nucleus, which is 4-6μm in size, spherical in shape and placed eccentrically (Kenneth, 2020). The nucleus has a clear membrane, the inner surface of which is lined with uniform and closely packed fine granules of chromatin; karyosome consists of several granules (Kenneth , 2020).

Cyst

During encystement the parasite is surrounded by a smooth, refractile non-staining wall which is about 0,5µm in thick. The size of the cyst varies between 12-15µm, the nucleus retains the characters of the trophozoite (Burton , 2015). Early in the development the cytoplasm of the cyst shows 1-4 chromatoid bars which are refractile, rounded end, the cyst is initially uninuclear but by binary fission soon develops into a binuclear and quadrinuclear body, as cyst matures both the glycogen mass and the chromadal bars generally disappear (Burton , 2015).

2.5.5 Life Cycle

The organism exists in two forms—the trophozoite and the cyst which is the dormant form. Human infection usually begins with the ingestion of the cyst which is present in food and/or water contaminated with human fecal material. Cysts survive the acidic pH of the stomach and pass into the intestine. In the ileo-cecal region, cysts undergo excystment and each cyst gives rise to eight trophozoites. These migrate to and multiply in the colon (Fotedal , 2017). Occasionally, however, trophozoites attack and invade the intestinal mucosa causing dysentery and/or progress through the blood vessels to extra-intestinal locations like liver, brain and lungs, where they may form life-threatening abscesses. In the intestine, many of the trophozoites encyst and produce quadrinucleated cysts (Biosci, 2014)

2.5.6 Clinical Symptoms

The incubation period is commonly 2-4 weeks but ranges from a few days to years. Amoebiasis is more severe in very young patients and in elderly patients. The clinical spectrum of amoebiasis ranges from asymptomatic infection to symptomatic (Lynne, 2017).

Asymptomatic Infection

In this state the patients have normal recto sigmoidoscopicfindings, without a history of blood in stool samples. Although people can be asymptomatically colonized withE. histolytica,they should be treated. Otherwise, some of these subjectsmay be dangerous environmentally or maydevelop colitis after a period of months (Mehmet and Petri, 2013).

Symptomatic Infection

Acute amoebic colitis, Amoebic dysentery is most common form of symptomatic invasive amoebiasis; seventy per cent of patients have a gradual onset of symptoms over 3 to 4 weeks after

infection, with increasingly severe diarrhea as primary complaint, accompanied by general abdominal tenderness (Ralph, 2004). The diarrhea is associated with pain in virtually 100 per cent of children, fever occurs in less than one half of the patients, abdomen distention and dehydration occur in fewer than 10 per cent of patients (Ralph, 2014). In young children, perforation, and peritonitis, or necrotizing colitis may develop rapidly (Ralph, 2014). The trophozoites penetrate the mucosa and sub mucosa of intestine and cause necrosis and form abscesses which later become fresh –shaped bleeding ulcers. In amoebic dysentery, the stool is acidic and contain mucous in which swarms of amoebae and blood corpuscles, are usually present (Bhamrah , 2012).

Extra intestinal localization, extra intestinal localization occurs, the liver is the most common site, but infection can also invade the lungs, pericardium, and brain. Symptoms may be acute or gradual and may include low-grade fever, pain in the light upper quadrant and weigh loss (Ortega, 2016).

2.5.7 Laboratory Diagnosis

Microscopic Stool Examination

Microscopic stool examination remains the main method for the diagnosis of amoebiasis and is used in most African countries However, it cannot differentiate between E. dispar and E. histolytica, and the accuracy of this method in detecting E histolytica depends heavily on the skills of the lab technician and has been shown to be less sensitive and less specific compared with other methods such as antigen detection(ELISA), and polymerase chain reaction (Nuruihasanah othman, 2019)

Culture, Xenic cultivation, first introduced by Boeck and Dr bohlav in 1925, is defined as the growth of the parasite in the presence of an undefined flora. This technique is still in use today using modified Locke-egg media (Haque et al, 2013). A xenic cultivation, first achieved in 1961, involves growth of the parasite in the absence of any other metabolizing cells. Cultures can be performed using fecal or rectal biopsy specimens and liver abscess aspirates. The success rate is between 50% and 70%, but the technique is technically difficult. Overall, it is less sensitive than microscopy (Nuruihasanah othman, 2019)

Antigen Detection, Enzyme-linked immunosorbent assay (ELISA) is used to detect antigens from E. histolytica in stool samples. Antigen-based ELISA kits using monoclonal antibodies against the GAL/GalNAc–specific lectin of E histolytica yield an overall sensitivity of 71%-

100% and specificity of 93%-100%. In patients with amebic liver abscess, serum and liver aspirate antigen detection using the same kit was shown to yield a sensitivity of 96% and 100%, respectively. Other stool detection kits use monoclonal antibodies against the serine-rich antigen of E histolytica or against other specific antigens. No specific antigen tests are available for the detection of E dispar and E moshkovskii from clinical samples (Nuruihasanah othman, 2019)

Serology, Multiple serologic assays are available for the diagnosis of amoebiasis. ELISA is the most used assay throughout the world and is used to measure the presence of serum antilectin antibodies (IgG). The sensitivity for detection of antibodies to E histolytica in patients with amebic liver abscess is 97.9%, whereas the specificity is 94.8%. False-negative results can occur within the first 7-10 days following infection (Mehmet and Petri 2013). Immunofluorescent assay (IFA) is also rapid, reliable, and reproducible. In the setting of amoebic liver abscess, the sensitivity and specificity of IFA was shown to be 93.6% and 96.7%, respectively. Indirect hemagglutination (IHA) is very specific (99.1%) but is less sensitive than ELISA. Counter-immunoelectrophoresis (CIE) is time-consuming but has shown a sensitivity of 100% in invasive amoebiasis . The seropositivity prevalence is very high in endemic areas, limiting antibody-based testing for diagnosing currently active disease, since antibodies can persist for years after (George R HEALY, 2020)

Polymerase Chain Reaction, E histolytica can be identified in various types of clinical specimens, including feces, tissues, and liver abscess aspirates. A wide variety of polymerase chain reaction (PCR) methods targeting different genes, including a small-subunit rRNA gene (18S rDNA), 30-kDa antigen gene, serine-rich protein gene, chitinase gene, hemolysin gene, and extrachromosomal circular DNA, have been described for the detection and differentiation of E histolytica, E dispar, and E moshkovskii (ROMANO NGUI, 2012)

2.5.8 Prevention

To help prevent the spread of amoebiasis around the home, washing of hands thoroughly with soap and clean water for at least 10 seconds after using the toilet or changing a baby's diaper, and before handling food is highly recommended , To enhance public health, the access to drinkable water must be accompanied at the same time by sanitation works and hygiene raising awareness among populations , Proper food hygiene is also among the effective ways of prevention of transmission of infection of E.histolytica (Aadish Rawat, 2020)).Good sanitary practice, as well as

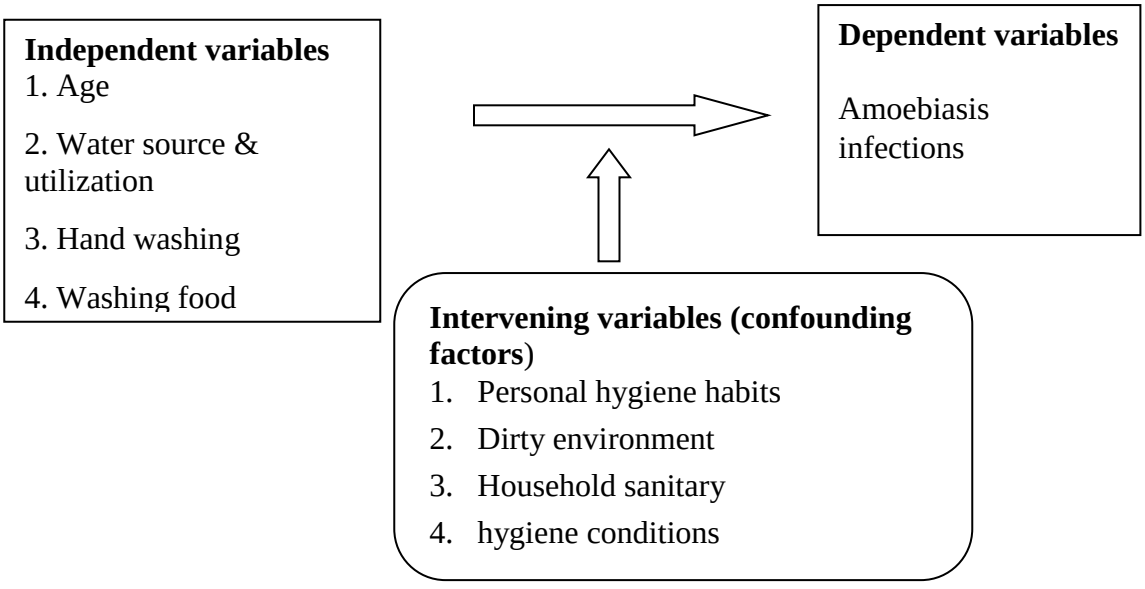
responsible sewage disposal or treatment, are necessary for the prevention of E.histolytica infection on an endemic level. Boiling and filtration of water supplies are necessary to reduce the incidence of infection (Aadish Rawat, 2020)).

2.5.9 Treatment of Amoebiasis

Treatment of amoebiasis includes the administration of both a luminal amoebicide for cysts and a tissue amoebicide for trophozoites. Common on treatment of amoebic liver abscess includes metronidazole (750mg or 35-50mg/kg every 8 hours for 7-10 days) or tinidazole (80mg or 60mg/kg 3times daily for 5 days) and luminal agent (Barceloux, 2018)

2.6 CONCEPTUAL FRAME WORK

This section shows the relationship between Independent variables, Dependent variables and intervening variables.



2.7 A RESEARCH GAP

There is a lacking of specific data on the prevalence, distribution, and disease burden of amoebiasis in Sub-Saharan Africa, despite the fact that limited sero-prevalence studies among pregnant women in SS Africa suggest that it may be common (Kamath, 2019). This study is being done to determine the prevalence of amoebiasis among pregnant patients at Mushubati Health center

CHAPTER THREE: METHODOLOGY

3.0 INTRODUCTION

This chapter includes the methodology which was used to carry out this study: research design, target population, sample design and data collection.

3.1 RESEARCH APPROACHES AND DESIGN

3.1.1 Research Approach

The data for this study was analysed using SPSS software, and the findings were validated using a quantitative research approach. Quantitative approach where emphasizing objective measurements and statistical analysis of data gathered through data collection sheet used. The study involved the statistical measurement (numbers) or numerical analysis of data.

3.1.2 Research design

This study was use retrospective design to assess the incidence of amoebiasis and associated risk factors among pregnant women attending Mushubati Health during 2022-2023. The study objectives were achieved through the use of administrative databases and medical information, using a retrospective methodology.

3.2 TARGET POPULATION

All pregnant women attending antenatal care attending Mushubati Health Center during 2022-2023 and requested the stool exam were the study's target group.

3.2.1 Inclusion criteria

In this study, pregnant women who attended at Mushubati Health Center because they were thought to have parasites were included.

3.2.2 Exclusion criteria

Women who requested other laboratory testing during pregnancy and those whose patient files were incomplete were excluded from this study.

3.2.3 SAMPLING PROCEDURES

Our sampling procedure strategy was convenience sampling, the participants was categorized into three classes according to the age group (18-25), (26-35) and (36-45),second class based on pregnancy trimester (first, second and third trimester) and the third class based on parity include (1 pregnancy, 2 pregnancies, 3 pregnancies and > 3 pregnancies.

3.2.4 Sample Size

To determine sample size, this study was used the model (Karlin & Taylor, 1981) where showing that a single population proportion formula that are used for determination of the sample size. The level of significance = 0.05, Marginal error is (e) = 0.05%, with 95% for confidence interval (CI). The sample size will be calculated by according to the following formula

$$n = \frac{N}{1+(N*e^2)} \quad \boldsymbol{n = \frac{195}{1+(195*0.05^2)} \; n = \frac{195}{1.77} = 110.1 \sim 110}$$

Where, n: sample size N: Total population e: Marginal error 1: Constant

3.2.5 Sampling Technique

To select hospitalized patients, a convenience sampling procedure was used. From those present, all patients admitted who meet the inclusion criteria was identified. Convenient sampling, also known as judgmental or subjective sampling, is a type of non-probability sampling in which researchers choose members of the population to participate in their surveys based on their own judgment (Etikan , 2016).

DATA COLLECTION TOOLS AND PROCEDURES

Following approval from the Mushubati Health Center research authorities as well as the Kibogora Polytechnic. The researchers contacted head of health center to explain the study's goal and methodology. Data was collected using structured data collection sheet and all data was retrieve into laboratory log book and in patient file in maternity and CPC register.

In our research we used so many instrument related with our qualification which were:

- Laptop

-Pen

-Paper

-Questioniare

3.4- DATA ANALYSIS

The statistical package for social sciences (SPSS) software, version 25, was used to compile, organize, filter, and analyze the data from this target population that was been collected, coded, and inputted. The descriptive statistic used to determine the frequencies, percentages, and contributing elements of high-quality healthcare delivery.

3.5 RELIABILITY AND VALIDITY MEASURES

The reliability of the tool was measured by the researcher with supervisor where structured data sheet for collecting data developed before data collection, the pilot study was performed. In this study, the content and construct validity was ensured when organizing items of the collection data sheet against the research objectives, the literature review and the conceptual framework.

3.6 ETHICAL CONSIDERATIONS

It is evident what plagiarism means and aware of the University's policy in this regard. The Institutional Review Board of Kibogora Polytechnic and the administrators of Mushubati Health Center must first give their approval before any data collection, though. The data's confidentiality and anonymity was upheld.

CHAPTER FOUR: DATA PRESENTATION, ANALYSIS, INTERPRETATION AND SUMMARY

4. 0. INTRODUCTION

This chapter involves the presentation, analysis and interpretation of findings in relation to the study objectives. It presents bio data of respondents and other data related to the study.

4.1 PRESENTATION AND INTERPRETATION OF THE RESULTS

Table 1: Distribution of participants according to their age group

Age Group	Frequency	Percent
18-25	17	15.45%
26-35	68	61.81%
36 -45	25	22.72%
Total	110	100%

Source: Mushubati HC 2022-2023

The pregnant women ranged in age from 18 to 45 years. The age group with the most women was 26-35 (61.81% (n=68), while the age group with the fewest was 18-25 (15.45% (n=17).

Table 2: Distribution of participants according to their pregnancy trimesters

Pregnancy trimesters	Frequency	Percent
First	29	26.36%
Second	63	52.27%
Third	18	16.36%
Total	110	100%

Source: Mushubati HC 2022-2023

According to Table 2, the second trimester of pregnancy had the highest participation rate (52.27% (n=63), followed by the first and third trimesters, which had lower participation rates of 26.36% (n=29) and 16.36% (n=18), respectively.

Table 3: Distribution of participants according to their parity

Parity	Frequency	Percent
1 pregnancy	15	13.63%
2 pregnancies	30	27.27%
3 pregnancies	25	22.72%
>3 pregnancies	40	36.36%
Total	110	100%

Source: Mushubati HC 2022-2023

According to table 3, the most common reported category of participant were pregnant women with more than 3 three pregnancies with 36.36% (n=40) followed by women with 2 pregnancies 27.27% (n=30), and women with 3 pregnancies with 22.72% (n=25).

Table 4: Prevalence of amoebiasis among pregnant women

AMOEBIASIS INFECTION		
Results	**Frequency**	**Percent**
Negative	22	20%
Positive (Entemoeba H)	35	31.81%
Other parasites	53	48.18%

Total	110	100%

Source: Mushubati HC 2022-2023

Amoebiasis was found in 31.81% of pregnant women (n=35). As shown in table 4, 31.81% (n=35) of participants were found to have amoebiasis, with 20% (n=22) of these being negative, while 50% (48.18% (n=53) of participants having other parasites.

Table 5: Assessment of risk factors associated with amobiasis among pregnant

Characteristic	Frequency	Percentage (%)	P-value	χ2 value
Washing food before cooking				
Yes	54	49	**.004**	3.566
No	56	51		
Cleaning kitchen materials				
Yes	36	32.72	**.028**	1.372
No	74	67.28		
Kitchen always clean				
Yes	48	43.63	.057	7.707
No	62	56.36		
Hand Washing after toilet				
Yes	40	36.36	**.036**	4.127
No	70	63.63		

Source: Mushubati HC 2022-2023

Table 5 above shows the factors associated with amoebiasis among pregnant women, where washing food before cooking accounted for 54%, cleaning kitchen materials with pipe water and detergent accounted for 36%, kitchen always cleaned accounted for 48%, and hand washing after

toilet accounted for 40%, all of which explain significance with regard to amoebiasis. Washing food before cooking Cleaning kitchen materials Hand Washing after toilet was statically significantly where P-value was <0.05

Table 6: Association of amoebiasis prevalence with water source

Characteristic	Frequency	Percentage (%)	P-value	χ2 value
Pipe water				
Yes	50	45.45	1.000	10.434
No	60	54.55		
Boiled water				
Yes	46	41.81	1.000	8.870
No	64	58.19		
Stagnant water				
Yes	62	56.36	.0000	7.078
No	48	43.64		
Mineral water				
Yes	30	27.27	1.000	6.833
No	80	72.73		

Source: Mushubati HC 2022-2023

Table 6 shows a relationship between the use of pipe water, boiled water, stagnant water, and mineral water usage and amobiasis, with 45.45%, 41.81%, 56.36%, and 27.27%, respectively. This could be due to infrastructure issues with producing clean water in the research location, as well as a lack of clean water habits among the locals. Usage of Stagnant water (P =.0000) was statically significantly for causing amoebiasis.

4.2 DISCUSSIONS OF FINDINGS

Parasitic infection(s), including amoebiasis, have been linked to an increased risk of pregnancy complications and poor outcomes in low-resource settings. Amoeba infections during pregnancy were linked to an increased risk of maternal complications and negative perinatal outcomes such as anemia, low birth weight, and perinatal mortality. In developing countries, elevated intestinal parasitic infections, including amoebiasis, have been observed due to poverty, low literacy rates, a lack of safe drinking water, poor hygiene, malnutrition, and a hot and humid tropical climate. The natural immune response to pregnancy makes pregnant women more susceptible to parasitic infections than non-pregnant women. Furthermore, intestinal parasitic infections disrupt both the maternal and fetal stages of pregnancy. The current study was conducted to determine the incidence of amoebiasis in pregnant women.

The overall prevalence of amoebiasis infection in the study was 31.18% (35/110) as shown in table 4 , this was high comparing by a study conducted by Damtie et al., on Prevalence and Associated Risk Factors of Intestinal Parasitic Infections among Pregnant Women Attending Antenatal Care in Yifag Health Center, Northwest Ethiopia where in their results they found the prevalence of amoeba among pregnant women was 9.4% (Damtie , 2021), also the prevalence of our study is high than the prevalence found in the study conducted by Amuta et al in Nigeria where they found a prevalence of amoebiasis of 18.9% among pregnant women studied in their study ((DERSOA, 2016)

According to the findings of our study, the prevalence of amoebiasis among pregnant women was lower compare to the study conducted by Supriya et al on Prevalence of intestinal parasites in pregnant women in India where the they find a prevalence of amoebiasis of 51.77% among pregnant women studied (Supriya , 2020), also was lower compare to the study of study by Alli on Prevalence of intestinal nematode infection among pregnant women attending antenatal clinic at the University College Hospital Ibadan Conducted in Nigeria Where their results were 43.4% among studied pregnant women (Alli , 2020).

Prevalence of amoebiasis was seen predominantly in second trimester with 12.72% followed by third trimester with 9.18% and first trimester with 9.09% trimester (Table5). Similar findings were reported in the study conducted by Alli et al., and Derso et al, as pregnancy increases there are

higher anti-inflammatory responses and lower proinflammatory responses. Progesterone is typically regarded as anti-inflammatory. Elevated concentrations of progesterone during 2nd trimester correlated with reduced activity of regulatory Th1 cells increases susceptibility to infections. By 3rd trimester anti-inflammatory responses are more elevated. This could be the reason for the greater prevalence of infections in the second and third trimester as compared to the first trimester (DERSOA, 2016)

In our investigation, a relationship was found between pipe water, cooked water, and stagnant water. This might be as a result of the infrastructure issue with water pipe manufacture in the research region as well as the lack of pipe water usage habits among the locals. Farmers use dangerous water to wash their equipment, their legs, and their hands after cultivating because there isn't a good canalization system for safe water. These findings are comparable to those of a study done in Nigeria by Addy et al., which found that individuals who used contaminated water were significantly more likely to contract amoebiasis, with a p value of 0.05 explaining the link between poor water hygiene and amoebiasis (DERSOA, 2016)).

Poor food handling hygiene and good food preparation have a greater impact on amoebiasis than main animal food consumption and plant consumption, which. This study can be contrasted with another one done in Accra by Ayeh-Kumi et al., where the main risk variables for amoebiasis was poor food hygiene. This is because food handlers in Accra are the source of amoebiasis while they are cooking (Ayeh-Kumi, 2019).

4.3 SUMMARY OF FINDINGS

Amoebiasis was discovered in 31.81% of pregnant women (n=35). According to the findings, 31.81% (n=35) of participants had amoebiasis, with 20% (n=22) being negative and 50% (48.18% (n=53) having other parasites. The study discovered that amoebiasis was more common in pregnant women between the ages of 18 and 25 (11.81%; n=13), 26 to 35 (13.63%; n=15), and 36 to 45 (6.36%; n=7). The prevalence of pregnancy in the second trimester was 12.72% (n = 14), while the prevalence in the first and third trimesters was 9.09% (n = 10) and 9.18% (n = 11), respectivel.

Washing food before cooking accounted for 54% of factors associated with amoebiasis among pregnant women, cleaning kitchen materials with pipe water and detergent accounted for 36%,

kitchen always cleaned accounted for 48%, and hand washing after toilet accounted for 40%, all of which explain significance with regard to amoebiasis. Amobiasis was associated with the use of pipe water, boiled water, stagnant water, and mineral water, with 45.45%, 41.81%, 56.36%, and 27.27%, respectively. This could be due to infrastructure issues in the research location, as well as a lack of clean water habits among the locals.

CHAPTER FIVE: CONCLUSION AND RECOMMENDATIONS

5.0 INTRODUCTION

In this chapter highlight the conclusion of our study, recommendation to those who interact with this problem.

5.1 CONCLUSION

The prevalence of amoebiasis was 31.18 percent (n=35) overall. Amoebiasis infection is a major problem among pregnant women in the study area. High parasitic infection is associated with poor hygienic and sanitation practices. Therefore, awareness creation through health education should be given to pregnancy on amoebiasis infection and associated factors.

Washing food before cooking Cleaning kitchen materials Hand Washing after toilet was statically significantly where P-value was <0.05. Usage of Stagnant water (P =.0000*) was statically significantly for causing amoebiasis.

5.2 RECOMMENDATIONS

It is recommended that:

i. Amoebiasis control can be implemented in the Rutsiro district, particularly in the Mushubati sector. This is because of the disease's high prevalence. The Ministry of Health or the health care services can do this.

ii. The government should provide residents with safe, clean water for domestic use in order to reduce the use of unsafe water sources such as dams/ponds, wells, and rivers.

iii. The use of safe water, such as piped water, should be promoted in the region, which can be done through community health education programs when patients visit the health center.

iv. Personal hygiene should be promoted in the region through government and non-governmental organization campaigns.

5.3 SUGGESTION FOR FURTHER STUDY

i. Molecular studies must be used to determine the true prevalence of Entamoeba histolytica in comparison to other Entamoeba species.
ii. Same study can be conducted by other researcher to reach most of population of Rustiro District
iii. A countrywide study should be done to have the real the status of this problem because this study used limited number of samples

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APPENDICES

APPENDIX 1: LETTER FOR GRANTING TO CONDUCT THE STUDY

KIBOGORA POLYTECHNIC

Kibogora, August 07th, 2023

Received and approved by
MUSHUBATI HC Manager
SINDIKUBWABO ANGELO.

To the Head of Mushubati Health Center

RE: Request of conducting Research

Dear sir,

We write this letter to humbly request to allow **Mrs. Gentille IHIMBAZWE and Mrs. Clemence TWAMBAZEMARIYA** to conduct research in your institution.

The above mentioned are bonafide students of Kibogora Polytechnic pursuing Bachelor's degree in Biomedical Laboratory sciences.

These students are currently conducting a research topic **"EVALUATE INCIDENCE OF AMOEBIASIS IN PREGNAT WOMEN ATTENDING MUSHIBATI HEALLTH CENTER DURING 2022 TO 2023"**

We are convinced that your institutionn will constitute a valuable source of information pertaining to their research, the purpose of this letter is to humbly request you to avail them the pertinent information they may need. we pledge to ensure that all provided information will be confidential and used in the strict academic purpose.

Any assistance rendered to the candidates will be highly appreciated.

Yours sincerely,

On behalf of KP Management

KIBOGORA POLYTECHNIC SCIENCE - FAITH - CONSCIENCE

Mr. NSENGIYUMVA Jean Paul
Ag. Dean of Health Sciences Faculty
Kibogora Polytechnic

APPENDIX 2: DATA SHEET FOR DATA COLLECTION

Code	Age	Pregnancy Trimester	Parity	Stool result

APPENDIX 3: QUESTIONNAIRE FOR DETERMINING THE RISK FACTORS

1. **Do you wash fresh food before cooking?**
   - Yes
   - No
2. **Do you wash your hands with soap after the toilet?**
   - Yes
   - No
3. **Do you wash your dishes and other kitchen items properly with pipe water and detergent?**
   - Yes
   - No
4. **Is your kitchen is always cleaned?**
   - Yes
   - No
5. **What kind of water do you typically drink or use?**
   - Pipe water
   - Boiled water
   - Stagnant water
   - Mineral water

Thank you!!!