CONTRIBUTION OF INTESTINAL PARASITES IN THE UNDER-FIVE DIARRHEA CASES IN BUBANZA PROVINCE, BURUNDI

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A DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN EPIDEMIOLOGY OF SOKOINE UNIVERSITY OF AGRICULTURE. MOROGORO, TANZANIA.

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EXTENDED ABSTRACT

A case-control study was conducted in the under-five children attending the Bubanza provincial hospital in Burundi from March to May 2021 to assess the contribution of selected intestinal parasites to diarrhea cases in the under-five children. A total of 340 stool samples were collected using cleaned cups, properly labelled and examined for intestinal parasites by direct microscopic examination using normal saline. Aliquots of remaining samples were preserved with 10% formalin and later examined by formal ether concentration technique and modified Ziehl-Nielsen method for diagnosis of *Cryptosporidium spp*. Socio-demographic data and risk factors were collected using a semi-structured questionnaire. Statistical analysis was done using SPSS version 21. Univariate and multivariate logistic regression used to compute the statistical association between positivity to intestinal parasites and independent variables including age of children, sex of children, marital status of the parents, source of income of the parents, education level of parents, source of water used, treatment of water, consumption of raw fruit, washing hands before consumption of fruit. A p-value less than 0.05 was considered as significant. Out of 340 children examined, 20.8% were detected in the study. About 17.3%, 1.7%, and 1.7% of the children were infested with *Entamoeba spp*, *Ascaris* and *Ancylostoma*, respectively. Multivariate regression model didn't reveal a significance in differences between the seasons (rain and dry season) in relation to the intestinal parasitic infestation (OR=11.2; 95% CI: 0.9-12; p = 0.05). Majority of the children attending the hospital without diarrhea were tested negative against intestinal parasitism (OR=0.08; 95% CI: 0.04-0.2; p = 0.00). This study found intestinal parasitic infestations to be highly prevalent in the study area. It is recommended to promote health education particularly to the parents / caregiver of the children, improving sanitation as well as promoting drinking

safe water in order to control and prevent intestinal parasite infestations. These methods were done to improve their health situation.

DECLARATION

I, Nyongera, Eliane do hereby declare to the Senate of Sokoine University of Agriculture that this dissertation is my original work done within the period of registration and that it has neither been submitted nor concurrently being submitted in any other institution.

20th May, 2022

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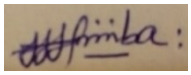

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DEDICATION

This dissertation is dedicated to my lovely husband, my parents and my family in general for their prayers during my studies.

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LIST OF ABBREVIATIONS AND SYMBOLS

^{0}C	Degree Celsius
CI	Confidence Interval
DHS	Demographic and Health Survey
ISTEEBU	Institut des Statistiques et d'Etudes Economiques du Burundi
IVAC	International Vaccin Access Center
KDHS	Kenya Demographic and Health Survey
MSPLS	Ministère de la Santé Publique et de lutte contre le Sida
OR	Odds Ratio
p	p-level
PCR	Polymerase Chain Reaction
RT-PCR	Reverse Transcription-Polymerase Chain Reaction
s	Second
SPSS	Statistical Package for the Social Sciences
UDHS	Uganda Demographic and Health Survey
UNICEF	United Nations International Children's Emergency Fund
WASH	Water Sanitation and Hygiene
WGO	World Gastroenterology
WHO	World Health Organization

CHAPTER ONE

1.0 GENERAL INTRODUCTION

1.1 Background Information of the Study

According to UNICEF/ WHO (2009), diarrhea is the condition of having at least three liquid or watery bowel movements each day. Worldwide, it remains one of the leading causes of mortality among the under-five children (UNICEF, 2018). Diarrhea kills around 2 195 children every day and 801 000 children deaths from diarrhea occur every year across the globe (IVAC, 2014; CDC, 2015). In Africa, Asia and South America, diarrhea accounts for one in eight deaths among children younger than five years per year (Keddy *et al*., 2016; Kotloff, 2017). Diarrhea accounts globally for approximately 8% (432 000) of all deaths in children under-five years old (IGME, 2018). Different prevalence of diarrhea in children under-five years of age was reported in 20% Mozambique (Mandomando *et al*., 2007), Kenya (Mulatya and Ochieng, 2020), Tanzania (Vargas *et al*., 2004) and in Uganda (UDHS, 2016).

The prevalence of diarrhea in Burundi ranges between 22% and 25% in under-five children with age-group between 6-23 months (ISTEEBU and MSPLS, 2010; ICF, 2012). Some factors responsible occurrence of diarrhea in the country are however, largely neglected by the research community. Diarrhea can be caused by several microorganisms including viruses, bacteria and protozoa (Platts-Mills *et al.,* 2015; Zhang *et al*., 2016). Some aetiologies of diarrhea cases in infants and under-five children are parasite infestations which include *Giardia*, *Cryptosporidium* and *helminthes* (WGO, 2012).

Among these intestinal parasites, *Cryptosporidium* is the most common aetiology, followed by *Giardia lamblia* and *Entamoeba histolytica* (Tellevik *et al*., 2015). All these three parasites can successfully be transmitted by water as a vehicle (Huang, 2006; Smith *et al*., 2007). Apart from diarrhea, other clinical symptoms associated with infestation of intestinal parasite include abdominal cramping, vomiting, flatulence, lower scores on a series of test of intelligence and weight loss.

Transmission of *Cryptosporidium* can be associated with drinking or swimming in contaminated water, eating uncooked or contaminated food that contain *Cryptosporidium* or by touching mouth with hands that had been in contact with a contaminated surface, object or animal (Strausbaugh and Herwaldt, 2000). According to the latest data published by WHO (2018), deaths associated with intestinal parasites in Burundi reached 10 058 or 11.98% of total death in under-five children. Studies on intestinal parasites have been done in different parts of East African countries and the prevalence of diarrhea in under-five children are reported to range between 15% and 40% (Ignatus *et al.*,2014; Mulatya and Ochieng, 2020). Exposure to diarrhea causing pathogens is conditioned by factors like age of the child, quality of water, availability of toilet facilities, level of education, household economic status, feeding practices and the general sanitary conditions hygiene (Stephen, 2005). Several outbreaks of diarrheal cases caused by *Cryptospordium parvum* have been reported during the last decade (Strausbaugh and Herwaldt, 2000). Abdominal cramping, vomiting, flatulence, weight loss and diarrhea are the main clinical manifestations of intestinal parasitic infestation.

1.2 Risk factors for Intestinal Parasitic Infestations

The common risk factors for intestinal parasitic infestations are poverty, poor hygiene and lack of access to potable water, food freshness, regular trimming fingernails, children

playground cleanliness and the family use of toilet (Mekonnen and Ekubagewargies, 2019). Ingestion of contaminated water, inadequate availability of water for hygiene and lack of access to sanitation are also known to contribute to deaths of 1.5 million children, where 88% of deaths are from diarrhea annually (WHO, 2002). Risk factors for intestinal parasitic infestation are grouped into three categories: a) Significantly associated, b) Non-associated and c) Non-consistently associated. Young age less than 23 months had been cited to be the predominant risk factor positively associated with all types of diarrhea (Ahmed *et al.*, 2008), whereas age less than 60 months has been found to be significantly positively associated with diarrhea by two studies (Manna *et al.*, 2004; Deshmukh *et al.*, 2009). Factors linked to mothers, such as literacy and occupation, may increase infestations of the child with intestinal parasites (WHO, 2002). However, the main risk factors for intestinal parasitic infestation in children under-five in Burundi are not documented.

1.3 Diagnosis for Intestinal Parasitic Infestations

Several tests can be used for detection of intestinal parasitic infestations. These include endoscopy which can be used to find parasites that can cause diarrhea, or other abdominal illnesses (Tellevik *et al*., 2015). This test is used when stool examination do not reveal the cause of diarrhea. Disadvantage of endoscopy is that it is very painful and also expensive than other test. Stool test can also be used to see if parasites are causing diarrhea. There are several tests that may be conducted on the stool sample. These include (a) stool culture: this examines stool for organisms that should not be present, or are present in too high numbers (CDC, 2020). Stool culture determines organisms that are present and if they could potentially be causing problems. (b) Ova and parasites tests: this test looks for signs of a parasite living in the intestinal tract (CDC, 2020). Stool samples can be examined under a microscope for parasites and their eggs. (c) Stool antigen tests: this

checks for antigens in the stool that may indicate the presence of parasites such as *Giardia spp., Cryptospordium spp.* and *Entamoeba histolytica* (CDC, 2020). (d) Blood smear which can be used to look for parasites that are found in the blood. This test is done by placing a drop of blood on microscope slide. (e) PCR test looks for the genetic fingerprint of a wide range of pathogens. RT-PCR assays for *Giardia spp., Cryptospordium spp.* and *Entamoeba histolytica* report high positive rate *Giardia lamblia, Cryptosporidium parvum* with 4.1%, while false positive results for *Entamoeba histolytica* will be avoided (CDC, 2020).

1.4 Prevention Measures and Treatment of Intestinal Parasitic Infestations

There are several measures for prevention of intestinal parasitic infestations including implementing water sanitation and hygiene (WASH) programs, which all aim at interrupting fecal oral transmission pathways, commonly referred to as the five ‘‘F’’ (fluids, fields, flies, fingers and food) (Wagner,1958). Diarrhea causing pathogens are usually spread via the faecal oral route through the consumption of food, or direct contact with infected stools. There is a need for urgent intervention such as poverty reduction programmes, preventive measure for ectoparasites, promotion of mass deworming, health education and accessing health care facilities as the major controlling and reducing transmission of intestinal parasite carriage where the prevalence is high (Nathan *et al*., 2018). Quigley *et al.* (2007) reported that exclusive breastfeeding plays a significant role in protecting children against common infectious agents during infancy, as well as preventing hospitalization for diarrhea. Diarrhea or other symptoms lasting for more than 2 to 3 days may necessitate medical attention (WHO, 2013).

1.5 Potential Complication of Intestinal Parasites

Many people are often able to fight against protozoan intestinal parasites. The intestinal parasites infestations can usually be resolved within a few weeks when the immune system is strong (Smith *et al*., 2007). However, people with weakened immune system like children under- five years can develop life-threatening complications from intestinal parasites. This includes severe dehydration, malnutrition, dangerous weight loss, spread to other areas of the body (Tellevik *et al*., 2015).

1.6 Problem Statement and Study Justification

Diarrhea is responsible of morbidity especially in children under-five years in Burundi (DHS, 2010). The prevalence of diarrhea in some areas of Bubanza (Buhurika and Gatura) had been estimated at 32.6% (Diouf *et al*., 2014). Despite, the burden that diarrhea causes to the health systems, there have been limited investigations on causes and factors responsible for its occurrence (Birmingham *et al*., 1997). The information from the study will provide insights on the possible contribution of parasitic infestations to diarrhea cases in children aged less than five years. The results from the study were expected to provide inputs to guide the scope of focus in investigating the causes and planning control strategies for the diarrhea syndrome in children.

1.7 Study Objectives

1.7.1 Main objective

General objective of this study was to assess the contribution and risk factors of selected intestinal parasites to diarrhea cases in the under-five children in Burundi.

1.7.2 Specific objectives

i. To quantify the magnitude of diarrhea in the under-five children attending at Bubanza provincial hospital.

ii. To establish the prevalence of intestinal parasites among diarrheal cases in under-five children at Bubanza provincial hospital.

iii. To identify risk factors associated with intestinal parasite infestation among diarrhea cases in the under-five children in Bubanza provincial hospital.

CHAPTER TWO

MANUSCRIPT 1

2.0 Contribution of intestinal parasites in the under-five-diarrhea cases in Bubanza province, Burundi

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2.1 Abstract

Background Information: Intestinal parasitic infestation is still a serious public health problem.

General Objectives: The main objective of the study was to assess the contribution and risk factors of selected intestinal parasite (*Entamoeba, Giardia* and *Cryptosporidium*) to diarrheic in under-five children attending Bubanza Provincial hospital in Burundi.

Methods and Methodology: Using matched case-control study, stool samples from 340 under-five children attending clinic were collected and examined for the presence of intestinal parasites using direct microscopic examination and normal saline methods (170 diarrheic and 170 non-diarrheic). Positive samples were preserved with 10% formalin and later examined by formal ether concentration technique and modified Ziehl-Nielsen method for detection of *Cryptosporidium spp*. In addition, parents/guardians of sampled children. Direct interview were used using semi-structured questionnaire to collect data related to socio-demographic information.

Results: From 340 stool samples collected and examined, 20.8% were found infected with at least one intestinal parasites. Detection of *Entamoeba histolyica* in diarrheic children (16.4%) was significantly higher than detection of *Entamoeba histolytica* in non-diarrheic (0.8%). Similarly, higher isolation rate of *Entamoeba coli* in diarrheic children (11.8%) was observed when compared to the isolation rate of *Entamoeba* coli in non-diarrheic (1.2%). Although, detection of *Ascaris* in diarrheic (2.4%) was also significantly higher than the proportion infested in non-diarrheic (1.2%). Detection of *ancylostoma* in diarrheic children was higher (2.9%) than the proportion infested in non-diarrheic (0.5%).

Conclusions: The study revealed that a higher proportion of under-five children with diarrhea had intestinal parasitic infestations compared to the non-diarrheic.

Keywords: Burundi; Children, Diarrhea; Intestinal; Parasites; under-five.

Article classification: Original research article

2.2 Introduction

Intestinal parasitic infestation is still a serious public health problem of the under-five children through the world particularly in African countries including Burundi. The parasitic infestation has reported to contribute to diarrhea cases, growing retardation, abdominal cramping, vomiting and weight loss [1]. Some aetiologies of parasite infestations isolated in diarrhea cases include *Giardia*, *Cryptosporidium* and helminthes [2]. Intestinal parasites can be transmitted by water as a vehicle [3]. Diarrhea remains one of the leading causes of mortality among the under-five children worldwide [4]. Different studies on intestinal parasites in under-five children have been carried out in African countries. In Kenya [5], Tanzania [6], Uganda [7] and Rwanda [8], studies reported prevalence ranging from14% - 40% respectively. Diarrhea is the third leading cause of morbidity and the fourth leading cause of mortality among children under-five years in Burundi [9]. Studies on prevalence of diarrhea in children under-five in Burundi are scarce. The prevalence of diarrhea in Burundi ranges between 22% and 25% in under-five children with highest prevalence reported in the 6-23 months age-group [10]. There is a need for urgent intervention such as poverty reduction programmes, preventive measure for parasitic infestation, promotion of mass deworming, health education and accessing health care facilities as the major controlling and reducing transmission of intestinal parasite carriage. Therefore, the objective of the study was to assess the contribution of selected intestinal parasites to diarrhea cases in the under-five children attending clinic at the Bubanza Provincial Hospital in Burundi.

2.3 Methodology

2.3.1 Study design and sample collection

A case-control study was conducted at Bubanza Provincial Hospital of Burundi from March 2021 to May, 2021 to assess the contribution of intestinal parasites in diarrhea cases in under-five children. The frequency of the participant enrollment was between 20 and 40 children per day. Participants were children under-five years of age, who during the study period were attending with diarrhea and other symptoms (fever, headache, vomiting) at this hospital. A total number of 340 children participated in the study. A matched case-control design was adopted with case to control ratio of 1:1 adopted. Therefore 170 children with diarrhea and 170 children without diarrhea were recruited by matching cases and control based on age and sex of participating child. Participation in the study was on voluntary basis and parents or guardians were requested to give consent to participate in the study to comply with the medical clearance requirements. Firstly, a stool specimen from each participating child was collected and examined for intestinal parasites. Secondary, direct interview based structured questionnaire was used to collect socio-demographic. The questionnaire was in English and translated in Kirundi (the native language). Children who were attending at Bubanza Provincial Hospital with other symptoms (fever, headache) and whose parents did not accept to participate in the study were excluded. The prevalence of diarrhea in some areas of Bubanza (Buhurika and Gatura) had been estimated at 32.6% [10]. Most of children attending Bubanza Provincial Hospital in the study were from Shari.

2.3.2 Data analysis

Collected data were entered into Microsoft Excel spread sheet for cleaning and then exported to SPSS version 21 for analysis. The prevalence of intestinal parasites was determined based on positivity of intestinal parasites during laboratory analysis. The

prevalence of intestinal parasites was computed as a proportion of positive samples from the total number of samples.

2.3.3 Ethical consideration

This study was approved by Medical ethical committee of the National Public Health Institute (CNE/08/2021) from Burundi and the Ministry of Public Health and the fight against AIDS (1697/CAB/2021).

2.4 Results

2.4.1 Demographic characteristics of under-five children attending clinic at Bubanza provincial Hospital

A Total of 340 under-five children attending the clinic, half of them were attending with diarrhea and other half were attending with other symptoms. Their age were in a range between 0 and 59 months. All groups of age were affected by intestinal parasites but children who were aged between 1 and 2 years were more affected compared to the group of age between 3 and 5 years. Fifty-five percent were male who were attending with diarrhea. Forty-one percent of their parents had informal educational. Majority of the parents (65%) had two children. Their source of income was mainly based on agriculture (90%) and 5% of them were employed (Table 1).

Variables	Class	Number of children with diarrhea n=170	Number of children without diarrhea n=170	Percent with diarrhea (%)	Percent without diarrhea (%)
Sex of children	Male	94	91	50.8	49.2
	Female	76	79	49.0	51.0
Age (month) of children	0-11	32	37	46.3	53.6
	12-23	57	45	55.8	44.1
	24-35	34	34	50.0	50.0
	36-47	28	38	42.4	57.5
	48-59	19	16	54.2	45.7
Marital status of the parents	Married	60	161	49.8	50.1
	Single	10	9	52.6	47.3
Number of under-five-children	1	69	55	55.6	44.3
	2	99	111	47.1	52.8
	3	2	4	33.3	66.6
Source of income of the parents	Agriculture	139	153	47.6	52.3
	Business	24	13	64.8	35.1
	Employed	1	2	33.3	66.6
	Unemployed	6	2	75	25
Educational level of the parents	Informal	67	89	44.2	57.0
	Primary	70	66	51.4	48.5
	Secondary	25	11	69.4	30.5
	Diploma	8	4	66.6	33.3

Table 1: Demographic characteristics of under-five children who participated in the study at Bubanza Provincial Hospital

Table 2: Prevalence of intestinal parasites in under-five children attending clinics at Bubanza provincial hospital during the period of the study

Parasites	Children attending clinic with diarrhea (n=170)	Percentage (%)	Children attending clinic without diarrhea (n= 170)	Percentage (%)
Entamoeba histolytica	36	21.2	1	0.6
Entamoeba coli	20	11.7	2	1.2
Ascaris spp.	4	2.4	2	1.2
Ancylostoma spp.	5	2.9	1	0.5

Detection of *Entamoeba histolyica* parasite in children with diarrhea 21.2%) was significantly higher than detection of *Entamoeba histolytica* in non-diarrheic children (0.8%) ($p<0.001$). Similarly, higher isolation rate of *Entamoeba coli* in children with diarrhea (11.8%) was observed when compared to the isolation rate of *Entamoeba*. coli in non-diarrheic children (1.2%) ($p<0.001$). On the other hand, detection of *Ascaris* in diarrheic children at 2.4 % was also significantly higher than the proportion infested in non-diarheic children (1.2%) ($p<0.001$). Although, detection of *ancylostoma* in diarrheic children was also higher (2.9) than the proportion infested in non- diarrheic children (0.5) ($p<0.001$).

Table 3: Mixed infestations of intestinal parasites in under-five children attending at Bubanza provincial hospital

Parasites	Under-five children attendin	Percentage (%)	Under-five children attendi	Percentage (%)

	g with diarrhea (n=170)		ng without diarrhea (n=170)	
Ascaris and Entamoeba	8	4.7	2	1.1
Ancylostoma and Entamoeba	6	3.5	1	0.5

Table 4: Distribution of intestinal parasites by age groups in the under-five children samples

Parasites	Age-groups (months)	Under-five children admitted with diarrhea n=170	Percentage (%)	Under-five children admitted without diarrhea n= 170	Percentage (%)
Entamoeba histolitica	0-< 3	0	0	0	0
	3-12	9	5.2	1	0.5
	>12-36	20	11.7	0	0
	>36-60	7	3.5	0	0
Entamoeba coli	0-<3	0	0	0	0
	3-12	3	1.7	0	0
	>12-36	13	7.6	0	0
	>36-60	4	2.3	2	1.1
Ascaris	3-12	0	0	0	0
	>12-36	1	0.5	1	0.5
	>36-60	1	0.5	0	0

		2	1.1	1	0.5
Ancylostoma	0-<3	0	0	0	0
	3-12	0	0	0	0
	>12-36	2	1.1	0	0
	>36-60	3	1.7	1	0.5

2.5 Discussion

This study investigated on the contribution of intestinal parasites to diarrhea cases among the under-five children attending clinic at the Bubanza Provincial Hospital in Burundi. The study revealed the presence of various intestinal parasitic infestations in under-five children. Our finding was different from a study conducted in Ethiopia which reported high prevalence (52%) than this study. The overall prevalence of intestinal parasites detected in this study was 20.8% in children attending with diarrhea which was different from the findings reported by previous authors in Mozambique [11] and Tanzania [6], who reported lower prevalence compared to this study. On the other hand, the prevalence of intestinal parasitic infestations reported from Ethiopia [12] and India [13] were higher than the proportion observed in the present study. However, all these studies indicated that intestinal parasites were common in children with diarrhea.

In this study, *Entamoeba* spp. (*Entamoeba histolytica* and *Entamoeba. coli*) were the most commonly detected parasites in under-five children and were followed by *Ancylostoma* and A*scaris. Cryptospordium spp and Giardia* were not detected in all specimens examined. Our findings are different from the report from Rome where *Giardia duodenalis, Cryptosporidium parvum* and *Entamoeba histolytica* were the most common protozoan parasites that caused acute diarrheal illnesses among under-five children The high prevalence reported in this study could be due to the ignorance of the parents regarding health and hygiene habits. In this study, there were no differences of intestinal parasitic infestation between seasons. Our results are different from the findings from Jordan [14] where most cases (62%) were found during rainy season. Also, findings of

this study are different from a report from Nigeria where more intestinal parasitic infestations were observed in the rainy season (17.6%) than the dry season [15]. The lack of association between occurrence of intestinal parasitic infestation and seasons could be to the small sample size used in this study.

Most of participants in the study had the same knowledge of intestinal parasite in general (Table 1) similar to the report from Ethiopia [16]. Results of the questionnaire survey in the study showed that the majority of the mothers were aware on the transmission mode of intestinal parasitic infestations similar to the report from Ethiopia [12]. Findings of this study indicated a significant number of the parents with informal education and their children had a high prevalence of parasitic infestations similar to the report from Mexico which revealed low maternal education levels. However, a study conducted in Turkey did not show any significant association between intestinal parasites in children and educational level of the parents [16]. This study indicated that under-five children who were attending the health facility without diarrhea (OR= 0.08, IC: 0.04-0.2) were likely more to be protected from intestinal parasitic infestations compared to the children who attended with diarrhea. In other words, under-five children attending with diarrhea to the provincial hospital were at high risk of getting intestinal and mixed parasitic infestations. This observation points out to a suggestion that intestinal parasites are involved as aetiologies of diarrhea.

Among the selected enteric protozoan pathogens for investigation in this study, *Entamoeba* spp. (*Entamoeba histolytica* and *Entamoeba coli*) were the most commonly detected organisms. The association of *Entamoeba* with diarrhea cases has been reported in a previous study in South Ethiopia [15]. The authors reported that, the organisms were of more frequent occurrence than others. Children with *Entamoeba histolytica* associated

with diarrheal illnesses are more likely to be malnourished Apart from *Entamoeba spp.*, other intestinal parasites namely *Ancylostoma* and A*scaris* were also detected in children presenting with diarrhea in this study. C*ryptosporidia* and *Giardia* were also among the selected intestinal parasites for investigation in the current study.

However, none of these parasites was detected from the children sampled during the period of the study. This situation could be explained by the program of administration of antiparasitic drugs to under-five children which is covered by the government every semester in the country. Contrary to our findings, a study in India [18] reported a prevalence of *Cryptosporidium* infestation in under-five children. *Entamoeba* were the most prevalent parasites detected in children under-five years regardless of their age and most of intestinal parasitic infestations were reported in male children which was a similar finding to the report from Ethiopia [19].

2.5.1 Study limitation

The time limitations and financial constraint influenced much on the purposive sampling in this study which affects the generalizability of our results.

2.5.2 Originality/Value

This study provides a significant result on the contribution of intestinal parasites in under-five diarrhea cases in Bubanza provincial hospital where many cases of diarrhea have at least one of the selected intestinal parasites.

2.6 Conclusion

The study revealed that a higher proportion of under-five children attending the clinic with diarrhea had intestinal parasitic infestations compared to the non-diarrheic counterparts.

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Conflict of interest

The authors declare that there is no conflict of interest for this study.

References

[1]. Kiani H, Haghighi A, Rostami A. Prevalence, risk factors and symptoms associated to intestinal.parasite infections among patients with gastrointestinal disorders in Nahavand. *Rev Inst Med Trop Sao Paulo*. 2016; 58(15): 42 - 65.

[2]. World Gastroenterology Organization [WGO]. Acute Diarrhea in Adults and Children, 2012*; A Global Perspective Milwaukee*, 24pp.

[3]. Huang, D. B. and White, A. C. An updated review on *Cryptosporidium* and *Giardia*. *Gastroenterology Clinics.* 2006; 35(2): 291 - 314.

[4]. United Nations Children's Fund and World Health Organization [UNICEF/WHO] Why children Are Still Dying and What Can Be Done.2018; New York. 68pp.

[5]. Mulatya, D. M. and Ochieng, C. Disease burden and risk factors of diarrhea in children under five years: Evidence from Kenya's demographic health survey. *International Society for Infectious Diseases.* 2020; 93(4): 359 - 366.

[6]. Vargas, M., Gascon, J., Casals, C., Schellenberg, D., Urassa, H., Kahigwa, E., Ruiz, J. and Vila, J. Etiology of diarrhea in children less than five years of age in Ifakara, Tanzania. *American Journal Tropical Medicine* Hygiene. 2004; 70(5): 536 - 539.

[7]. Uganda Bureau of Statistics Kampala and Uganda Demographic and Health Survey. Key indicators. 2016. 169pp.

[8]. Ignatus, R., Gahutu, J. B., Klotz, C., Steininger, C., Shyirambere, C., Lyng, M. and Mockenhaupt, F. P. High prevalence of *Giardia duodenalis* assemblage B infestation and association with underweight in Rwandan children. *PLoS Neglected Tropical Diseases*. 2012; 6(6): 16 - 27.

[9]. Institut de Statistiques et d'Études Économiques du Burundi [ISTEEBU], Ministère de la Santé Publique et de la Lutte contre le Sida [Burundi] [MSPLS], et ICF International (2012). *Demographic Health Survey* [DHS, ISTEEBU, MSPLS, ICF] International, Bujumbura, Burundi. 2010; 419pp.

[10]. Diouf, K., Tabatabai, P., Rudolph, J. and Marx, M. Diarrhea prevalence in children under five years of age in rural Burundi: an assessment of social and behavioral factors at the household level. *Global Health Action.* 2014; 7(1): 24 - 35.

[11]. Mandomando, I. M., Macete, E.V., Ruiz, J., Sanz, S., Abacassamo, F. and Vallès, X. Etiology of diarrhea in children younger than 5 years of age attending in a rural hospital of Southern Mozambique. *American Journal Tropical Medicine Hygiene.* 2007; 76 7): 522 - 525.

[12]. Beyene, G. and Haile-Amlak, A. Antimicrobial sensitivity Pattern of Campylobacter species among children in Jimma University Specialized Hospital, Southwest Ethiopia. *Ethiopian Journal of Health Development.* 2004; 19(1): 185 - 186.

[13]. Youngsie, H. B. N. Pathogenic microorganisms associated with childhood diarrhea in Low-and-middle income countries: Case study of Yaoundé – Cameroon. *International Journal of Environmental Research Public Health.* 2008; 5: pp. 213 - 229.

[14]. Jaran, A. S. Prevalence and seasonality variation of human intestinal parasites in patients attending hospital with abdominal symptoms in Northern Jordan. *Eastern Mediterranean Health Journal*. 2016; 22(10): 756 - 760.

[15]. Frederick, O. A., Christopher, E. O. and Richard, O. Seasonal Variation of intestinal parasitic infestations among HIV positive patients in Benin City, Nigeria. *Ethiopian Journal of Health Sciences*. (2011); 24(5): 9 - 20.

[16]. Mulatu, G., Zeynudin, A., Zemene, E., Debalke, S. and Beyene, G. Intestinal parasitic infestations among children under five years of age presenting with diarrheal diseases to two public health facilities in Hawassa, South Ethiopia. *Infectious Diseases of Poverty.* (2015); 4(1): 49 - 51.

[17]. Okyay, P., Ertug, S., Gultekin, B., Onen, O. and Beser, E. Intestinal parasites prevalence and related factors in school children, a western city sample- Turkey. *BioMed Central Pub Health*. (2004); 4(1): 64 - 69.

[18]. Kaur, R., Rawat, D., Kakkar, M., Uppal, B. and Sharma, V. K. Intestinal parasites in children with Diarrhea in Delhi, India. *Southeast Asian Journal of Tropical Medicine and Public Health.* (2002); 725 - 729.

[19]. Eshetu, G. and Kefiyalew, J. Prevalence and factors associated with intestinal parasitic infestation among under-five children in and around Haro Dumal Town, Bale zone, Ethiopia. Federal Democratic Republic of Ethiopia. *National Hygiene and Sanitation Strategy Ministry of Health*. 2018; 85pp.

CHAPTER THREE

MANUSCRIPT TWO

3.0 Contribution of intestinal parasites in the under-five-diarrhea cases in Bubanza province, Burundi

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3.1 Abstract

3.1.1 Background

Diarrhea remains one of the leading causes of mortality among the under-five children, worldwide. However, there is a little information on the contribution of intestinal parasite in the under-five children in Burundi. This study was carried to assess the contribution and risk factors of selected intestinal parasite (*Entamoeba*, *Giardia* and *cryptosporidium*) to diarrheic in under-five children attending Bubanza Provincial hospital in Burundi. A matched case-control study and interview based on structured questionnaire was conducted. From 340 under-five children attended the clinic (170 attended with diarrhea and 170 were attended without diarrhea), Sample were collected and examined for the presence of intestinal parasites using direct microscopic examination and normal saline methods. Positive samples were preserved with 10% formalin and later examined by formal ether concentration technique and modified Ziehl-Nielsen method for detection of

Cryptosporidium spp. From stool samples examined, 20.8% were found infected with at least one intestinal parasite. Detection of intestinal parasite in under-five children was significantly higher (17.6%) than detection of intestinal parasite in non-diarrhea cases (3.2%). The study indicated that the under-five children who attended the hospital without diarrhea were more likely to test negative for intestinal parasitic infestations compared to the children attended with diarrhea.

Key words: Intestinal parasites, Diarrhea, under-five, Children, Burundi

3.2 Introduction

Diarrhea is one of the symptoms of intestinal parasite caused by waterborne infestations or non-infectious condition which is reported to cause a significant number under deaths in under-five children (Mengistie *et al.,* 2013). Its remains one of the leading causes of mortality among the under-five children Worldwide. (UNICEF, 2018). In Africa, Asia, and South America, diarrhea accounts for one in eight deaths among children younger than five years per year (Keddy *et al*., 2016; Kotloff, 2017). Different studies on intestinal parasites in under-five children have been carried out in African countries .In Kenya (Mulatya and Ochieng, 2020), Tanzania (Vargas *et al*., 2004), Uganda, (UDHS). Studies reported the prevalence ranging from 14% - 40% respectively. Some aetiologies of diarrhea cases in infants and under-five children are parasite infestations which include *Giardia*, *Cryptosporidium* and *helminthes* (WGO, 2012). Intestinal parasites can be transmitted by water as a vehicle (Huang, 2006; Smith *et al*., 2007). In Burundi, there is little information on contribution of intestinal parasites to morbidity and mortality associated with diarrhea particularly in the under-five children. Studies on prevalence of diarrhea in children under-five in Burundi are scarce. The prevalence of diarrhea in Burundi ranges between 22% and 25% in under-five children with highest prevalence reported in the 6-23 months age-group (Diouf *et al.,* 2014). There is a need for urgent

intervention such as preventive measure for parasitic infestation, promotion of mass deworming, health education and accessing health care facilities as the major controlling and reducing transmission of intestinal parasite carriage. Therefore, this study was conducted to assess the contribution of selected intestinal parasites to diarrhea cases in the under-five children attending clinic at the Bubanza Provincial Hospital in Burundi (Figure 1).

Figure 1: Map of Bubanza provincial hospital (UNOCHA, 2018).

3.3 Materials and Methods

3.3.1 Study area

Bubanza provincial hospital located in Northwestern part of Burundi. In 2019, the population of Bubanza Province was 462 819 inhabitants, with population density of 425 /km^2. Bubanza province has a warm climate with average temperature of 27^{0}C.

3.3.2 Study design

We conducted a case-control study at Bubanza Provincial Hospital of Burundi from March-May 2021 to assess the contribution of intestinal parasites in diarrhea cases in under-five children. The frequency of the participant enrollment was between 20 and 40 children per day.

3.3.3 Sampling

A total of 340 under-five children participated in the study including those with diarrhea (n =170 children) and those without diarrhea (n=170 children), whose parents/guardians accepted to participate in the study. Before sampling, participants were recruited starting by an introduction of the investigator, then explaining the general objective of the study. They were informed and ensured about the confidentiality of their answers and their identities. For each occasion, participants who were attending to this provincial hospital and who gave their consent to participate in the study were also informed about the importance of this research. Children who were attending at Bubanza provincial hospital with other symptoms (fever, headache) and whose parents did not accept to participate in the study were excluded. Participation in the study was on voluntary basis.

3.3.4 Sample size estimation

The sample size obtained was calculated by using the formula: $n=Z^2*P*(1-P)/d^2$. Source: (Diouf *et al.*, 2014).

Where, n= Sample size; Z = Z statistic, determined based of confidence level, Confidence interval $\alpha=¿$ 1.96; P = expected prevalence of diarrhea in under-five years and d = Precision (5%). After calculation, 340 under-five children were found, where n = 170 children were attending clinic with diarrhea and n = 170 children were attending without diarrhea. The frequency of the participant was between 20 and 40 children per day.

3.3.5 Data collection

A stool specimen from each participating under-five children attending the clinic during the period of the study was collected and examined for intestinal parasites. Participants were children under-five years of age, who during the study period were attending with diarrhea and other symptoms (fever, headache, vomiting) at Bubanza provincial hospital. Direct Interview was used to collect socio-demographic using questionnaire. The questionnaire was in English and translated in Kirundi (the native language). Children who were attending at Bubanza Provincial Hospital with other symptoms (fever, headache). Excluded the children whose their parents did not accept to participate in the study.

3.3.6 Data management and analysis

Collected data were entered into Microsoft Excel spread sheet for cleaning and then exported to SPSS version 21 for analysis. The prevalence of intestinal parasites was determined based on positivity of intestinal parasites during laboratory analysis. The prevalence of intestinal parasites was computed as a proportion of positive samples from the total number of samples. Independent variable was screened by unvariable logistic regression analysis for their association with the positivity to intestinal parasites. Multivariate logistic regression analysis was used to determine the association between risk factors with the positivity to intestinal parasites by using backward Wald methods. A variable with a *P*-value less than 0.05 was considered significant. Odds ratios were determined with 95% confidence interval.

3.3.7 Ethical consideration

This study was approved by Medical ethical committee of the National Public Health Institute (CNE/08/2021) from Burundi and the Ministry of Public Health and the fight against AIDS (1697/CAB/2021).

3.4 Results

3.4.1 Demographic characteristics of under-five children attending clinic at Bubanza provincial Hospital

A total of 340 children under-five years were involved during the period of the study, where Half of the children attended clinic were with diarrhea (n= 170 under-five children) and the other half (n= 170 under-five children) were without diarrhea. Their age were ranged between 0 and 59 months. All groups of age were affected by intestinal parasites but children who were aged between 1 and 2 years got more prevalence compared to the other groups (Table 1). Fifty-five percent were male who were attending with diarrhea. Forty-one percent of their parents had informal educational. Majority of the parents had two children (65%), agriculture was the main source of income.

Table 1: Demographic characteristics of under-five children attending clinic at Bubanza provincial Hospital

Variables	class	Under-five children attending with diarrhea n=170	Under-five children attending without diarrhea n=170	Under-five children attending with diarrhea (%)	Under-five children attending without diarrhea (%)
Sex of children	Male	94	91	55.2	53.5
	Female	76	79	44.8	46.5
Age (months) of children	0-11	32	37	18.8	21.7
	12-23	57	45	31.7	26.4
	24-35	34	34	20.0	20.0
	36-47	28	38	16.4	22.3
	48-59	19	16	11.1	9.4
Marital status of the parents	Married	160	161	94.1	94.8
	Single	10	9	5.9	5.2
Number of children under-five year in household	1	69	55	40.6	32.3
	2	99	111	58.2	65.2
	3	2	4	1.2	2.3
Source of income of the parents	Agriculture	139	153	81.7	90.0
	Business	24	13	14.1	7.6
	Employed	1	2	0.6	1.2
	Unemployed	6	2	3.6	1.2
Educational level of the parents	Informal	67	89	39.4	52.3
	Primary	70	66	41.1	38.9
	Secondary	25	11	14.7	6.4

Table 2: Knowledge of the parents of under-five children about diarrhea and intestinal parasites infestations

Variables	Responses	Participant whom their children were diarrheic	Participants whom their children were non diarrheic	Signification p-value)	N = 40	%
Diarrhea definition	Going on toilet more than 3times/day	92	100	0.6	192	56.4
	Loosing something like water	23	26		49	14.4
	All the above	55	44		99	1.4
Parents hearing about diarrhea	Yes	168	170	0.1	338	99.4
	No	2	0		2	0.6
Ways of experiencing diarrhea	On my Self	12	13	0.6	25	7.3
	On my children	105	94		199	58.5
	Hospital	7	12		19	5.5
	By family	22	22		44	12.9
	Radio	24	29		53	15.5
Age group tovulnerable intestinal parasites	7-35months	169	166	0.5	335	98.2
	36-59months	1	4		5	
Measures for preventing intestinal parasites	I don't know	5	7	0.2	12	3.5
	Practicing good	108	901		198	58.2

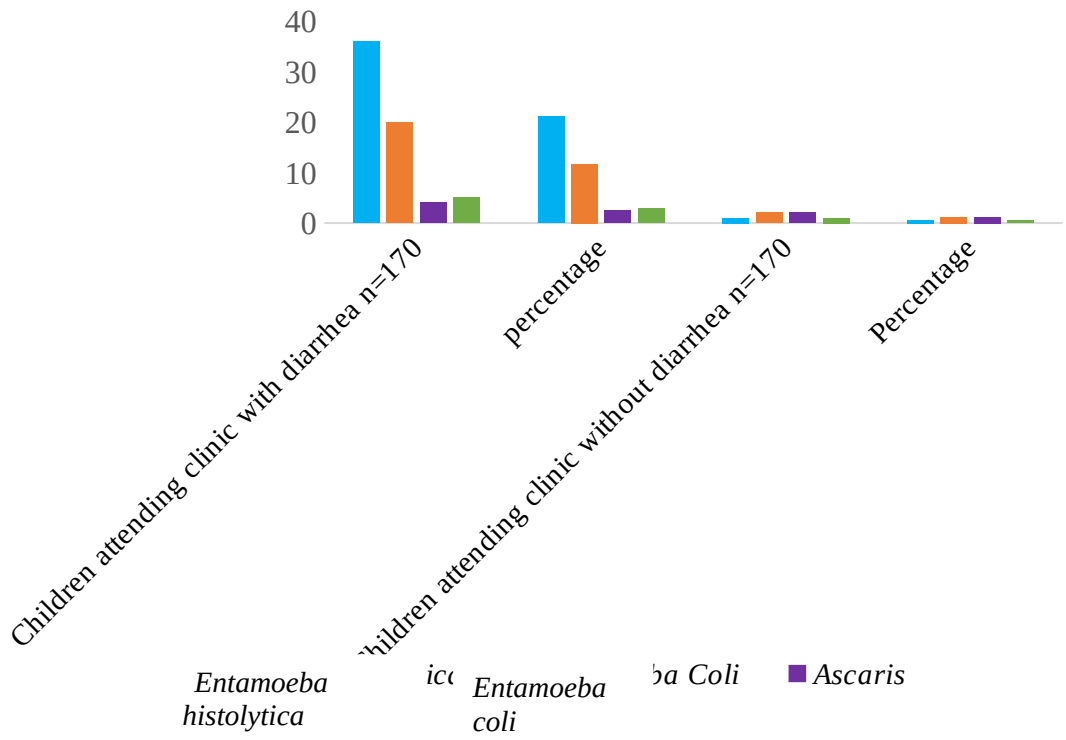

Figure 2: Prevalence of intestinal parasites in under-five children attending clinics at Bubanza provincial hospital during the period of the study

The report from this study show that detection of *Entamoeba histolytica* parasite in children attending with diarrhea was significantly higher (21.2%) than detection of *Entamoeba histolytica* in children attended without diarrhea (0.6%) ($p<0.001$). Similarly, higher isolation rate of *Entamoeba coli* in children attended without diarrhea (11.8%) was observed when compared to the isolation rate of *Entamoeba coli* children attended with diarrhea (1.2%) ($p<0.001$). On the other hand, detection of *Ascaris* in diarrheic children at (2.4 %) was also significantly higher than the proportion infested in children attended

without diarrhea (1.2%) (p<0.001). Although, detection of *Ancylostoma* in children attended with diarrhea was also higher (2.9%) than the proportion infested in non-diarrheic children (0.6) (p<0.001).

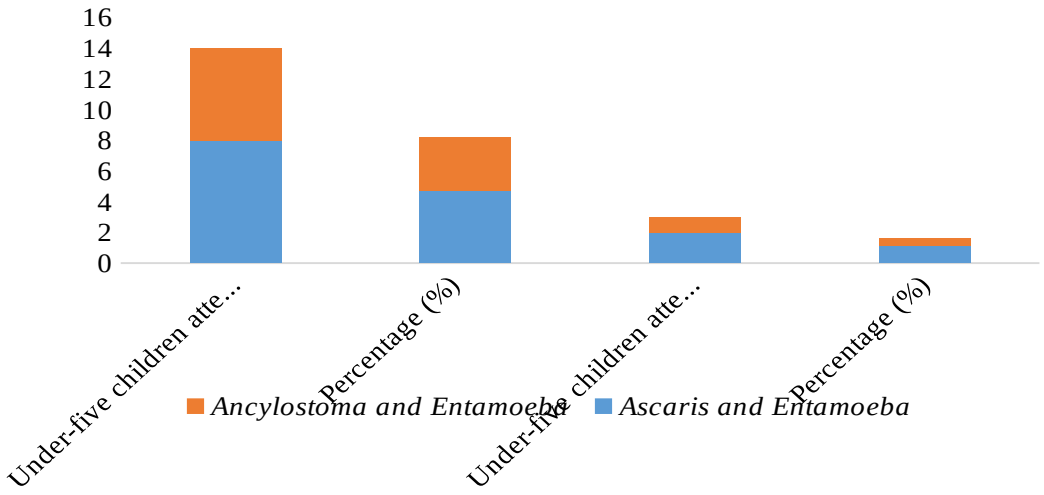

Figure 3: Mixed infestations of intestinal parasites in under-five children attending at Bubanza provincial hospital

Table 3: Distribution of intestinal parasites by age groups in the under-five children samples

Parasites	Age-groups (months)	Under-five children admitted with diarrhea n=170	Percentage (%)	Under-five children admitted without diarrhea n= 170	Percentage (%)
Entamoeba histolytica	0-< 3	0	0	0	0
	3-12	9	5.2	1	0.5
	>12-36	20	11.7	0	0
	>36-60	7	3.5	0	0
Entamoeba coli	0-<3	0	0	0	0
	3-12	3	1.7	0	0
	>12-36	13	7.6	0	0
	>36-60	4	2.3	2	1.1
Ascaris	0-<3	0	0	0	0
	3-12	1	0.5	1	0.5
	>12-36	1	0.5	0	0
	>36-60	2	1.1	1	0.5
Ancylostoma	0-<3	0	0	0	0

3.5 Discussion

This study investigated on the contribution of intestinal parasites to diarrhea cases among the under-five children attending clinic at Bubanza Provincial Hospital in Burundi. *Entamoeba* spp. (*Entamoeba histolytica* and *E. coli*) were the most commonly detected parasites in under-five children in the study particularly in children attended with diarrhea. *Cryptospordium spp and Giardia* were not detected in all examined specimen. Children aged between 1 and 2 years got more prevalence of intestinal parasites infestations compared to children between 3 and 5 years (Table 3). The overall prevalence of intestinal parasites detected in this study was 20.8% in children attending the clinic which was different from the findings reported by previous studies in Kenya (KDHS, 2007) and Tanzania (Vargas *et al.*, 2004), who reported lower prevalence's compared to this study. On the other hand, the prevalence of intestinal parasitic infestations reported from Ethiopia (Beyene *et al.*, 2004) and India (Youngsie, 2008) were higher than proportion observed in the present study.

However, all these studies indicated that intestinal parasites were common in children with diarrhea compared to the children attending without diarrhea while the prevalences for *Ancylostoma* and A*scaris* were equal in the study. Results of the questionnaire survey in the study showed that the majority of the mothers were aware on the transmission mode of intestinal parasitic infestations similar to the report from Ethiopia (Beyene *et al*., 2014). Findings of this study indicated a significant number of the parents with informal education and their children had a high prevalence of parasitic infestations similar to the report from Mexico which revealed low maternal education levels (Quihui, 2006). Under-five children attending with diarrhea to the provincial hospital were at high risk of tested positive to the intestinal parasite and mixed parasitic infestations were occurred in the study. This observation points out to a suggestion that intestinal parasites are involved as

aetiologies of diarrhea. The time limitations and financial constraint influenced much on the purposive sampling in this study which affects the generalizability of our results. There are also some parents with under-five children attending with other symptoms (fever, headache) during the period of the study and did not accept to participate in the study.

3.6 Conclusion

This study aimed to assess the contribution of intestinal parasites in diarrhea cases among children under-five year of age. It also aimed at establishing the risk factors associated with intestinal parasites in the sampled children. Generally, the study revealed that a higher proportion of under-five children attending the clinic with diarrhea had intestinal parasitic infestations compared to the non-diarrheic counterparts. The investigations targeting to establish the cause of diarrhea in clinical medicine as well as the research should include intestinal parasites in the list. Furthermore, efforts to reduce childhood illnesses should focus on educating women on hygiene promotion for all age groups especially children under- five year.

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References

Beyene, G. and Haile-Amlak, A. (2004). Antimicrobial sensitivity Pattern of Campylobacter species among children in Jimma University Specialized Hospital, Southwest Ethiopia. *Ethiopian Journal Health Development* 19(1): 185 - 186.

Diouf, K., Tabatabai, P., Rudolph, J. and Marx, M. (2014). Diarrhea prevalence in children under- five years of age in rural Burundi: an assessment of social and behavioral factors at the household level. *Global Health Action* 7(1): 24 - 35.

Huang, D. B. and White, A. C. (2006). An updated review on *Cryptosporidium* and *Giardia*. *Gastroenterology Clinics* 35(2): 291 - 314.

Keddy, K. H., Smith, A. M. and Page, N. A. (2016). GEMS extend understanding of childhood diarrhea. *Lancet* 388(10051): 1252 - 1254.

Kotloff, K. L. (2017). The burden and aetiology of diarrheal illness in developing countries. *Pediatric Clinical* 64(4): 799 - 814.

Mengistie, B., Berhane, Y. and Work, A. (2013). Prevalence of diarrhea and associated risk factors among children under-five years of age in Eastern Ethiopia: A cross-sectional study. *Open Journal of Preventive Medicine* 3(07): 446 - 453.

Mulatya, D. M. and Ochieng, C. (2020). Disease burden and risk factors of diarrhea in children under-five years: Evidence from Kenya's demographic health survey. *International Society for Infectious Diseases* 93: 359 - 366.

Quihui, L., Valencia, M. E., Crompton, D.W.T., Phillips, S., Hagan, P. and Morales, G. (2006). Role of the employment status and education of mothers in the

prevalence of intestinal parasitic infestations in Mexican rural school children. *Bio Medical Central Pub Health* 6: 225 - 232.

Smith, H. V., Caccio, S. M., Cook, N., Nichols, R. A. B. and Tait, A. (2007). *Cryptosporidium* and *Giardia* as foodborne zoonoses. *Veterinary Parasitology* 149(2): 29 - 40.

Uganda Bureau of Statistics Kampala and Uganda Demographic and Health Survey (2016) Key indicators 169pp.

UNICEF (2018). [http://www.data.unicef.Org/topic/child-health/diarrhea-disease]. Site visited on 20/5/2018.

UNOCHA (2018). United Nations Office for the Coordination of Humanitarian Affairs in Burundi. [http://www.unocha.org/infominghumanitarianswordwide.]. Site visited on 25/5/2022.

Vargas, M., Gascon, J., Casals, C., Schellenberg, D., Urassa, H., Kahigwa, E., Ruiz, J. and Vila, J. (2004). Etiology of diarrhea in children less than five years of age in Ifakara, Tanzania. *American Journal of Tropical and Medicine Hygiene* 70(5): 536 - 539.

WGO (2012). Acute Diarrhea in Adults and Children. *A Global Perspective Milwaukee*. World Gastroenterology Organization, 24pp.

Youngsie, H. B. (2008). Pathogenic microorganisms associated with childhood diarrhea in Low-and-middle income countries: Case study of Yaoundé – Cameroon. *International Journal of Environment Research and Public Health* 5: 213 - 229.

CHAPTER FOUR

4.0 GENERAL DISCUSSION

Generally, the study investigated the contribution of intestinal parasites to diarrhea cases among the under-five children in Bubanza Province of Burundi. The results of the study revealed the presence of various intestinal parasitic infestations in under-five children. In this study, children aged between 1 and 2 years presented with higher prevalence of intestinal parasites infestations compared to children between 3 and 5 years (Table 3). Our finding was different from a study conducted in Ethiopia which reported higher prevalence of (52%) comparer to this study (Eshetu and Kefiyalew, 2018). The overall prevalence of intestinal parasites detected in this study was 20.8% in children attending the hospital with diarrhea which was different from the findings reported by previous authors in Mozambique (Mandomando *et al.,* 2007); Kenya (KDHS, 2007) and Tanzania (Vargas *et al.,* 2004), who reported lower prevalences compared to this study. On the other hand, the prevalences of intestinal parasitic infestations reported in Ethiopia (Beyene *et al.*, 2004) and India (Youngsie, 2008) were higher than proportion observed in the present study. However, all these studies indicated that intestinal parasites were common among children with diarrhea.

In this study, *Entamoeba* spp. (*Entamoeba histolytica* and *E. coli*) were the most commonly detected parasites in under-five children. The prevalence of *Ancylostoma* and A*scaris* were equal in this study. *Cryptospordium spp and Giardia* were not detected in all examined specimen. Our findings are different from the report from Rome where *Giardia duodenalis*, *Cryptosporidium parvum* and *Entamoeba histolytica* were the most common protozoan parasites that caused acute diarrheal illnesses among under-five

children (Sperber *et al.*, 2012). The high prevalence reported in this study could be due the ignorance of the parents regarding health and hygiene habits.

In this study, there were no differences of intestinal parasitic infestation between seasons (Here, we were compared when intestinal parasite were very common Compared to the seasons (rain season and dry season). Our results are different from the findings from Jordan (Jaran, 2016) where most cases (62%) were found during rainy season. Also, from Nigeria where more intestinal parasitic infestations were observed in the rainy season (17.6%) than the dry season (Frederick *et al.*, 2011). The lack of association between occurrence of intestinal parasitic infestation and seasons could be due to the small sample size used in this study.

Most of participants in the study had the same knowledge of intestinal parasite in general (Table 2) similar to the report from Ethiopia. Results of the questionnaire survey in the study showed that the majority of the mothers were aware on the transmission mode of intestinal parasitic infestations similar to the report from Ethiopia (Beyene *et al.*, 2014). Findings of this study indicated a significant number of the parents with informal education and their children had a high prevalence of parasitic infestations similar to the report from Mexico which revealed low maternal education levels (Quihui, 2006).

However, a study conducted in Turkey did not show any significant association between intestinal parasites in children and educational level of the parents (Okyay, 2004). This study indicated that under-five children who were attending the health facility without diarrhea (OR= 0.08, IC: 0.04-0.2) were more protected from intestinal parasitic infestations compared to the children attending with diarrhea. In other words, under-five children with diarrhea attending to the provincial hospital were more prevalent of intestinal parasite and mixed parasitic infestations were found compared to those

presented without diarrhea. This observation points out to a suggestion that intestinal parasites are involved as aetiologies of diarrhea. Among the selected enteric protozoan pathogens for investigation. In this study, *Entamoeba* spp. (*Entamoeba histolytica* and *Entamoeba coli*) were the most commonly detected organisms. The association of *Entamoeba* with diarrhea cases has been reported in a previous study in South Ethiopia (Mulatu *et al.*, 2015). Apart from *Entamoeba spp.*, other intestinal parasites namely *Ancylostoma* and A*scaris* were also detected in children presenting with diarrhea in this study.

C*ryptosporidia* and *Giardia* were also among the selected intestinal parasites for investigation in the current study. However, none of these parasites was detected from the children sampled during the period of the study. This situation could be explained by the program of administration on antiparasitic drugs to under-five children which are covered by the government every semester. Contrary to our findings, a study in India (Kaur, 2002) reported a prevalence of *Cryptosporidium* infestation in under-five children. *Entamoeba* were the most prevalent parasites detected in children under-five years regardless of their age and most of intestinal parasitic infestations were reported in male children which was a similar finding to the report in Ethiopia (Eshetu and Kefiya, 2018). There was no significant association found in the study between some risk factors (source of water, habit of treated water used) and parasitic infestation, this differs from findings of the report from Egypt (Mahfouz *et al*., 1997).

4.1 Conclusion and Recommendations

The present study aimed to assess the contribution of intestinal parasites in diarrhea cases among children under-five year of age. It also aimed at establishing the risk factors associated with intestinal parasites in the sampled children. The study revealed that a

higher proportion of under-five children attending clinic with diarrhea had intestinal parasitic infestations compared to the non-diarrheic counterparts.

This is suggestive of the involvement of these parasites as aetiologies of diarrhea. The study also indicates that under-five children who attended the hospital without diarrhea were more likely to test negative for intestinal parasitic infestations. The investigations targeting to establish the cause of diarrhea in clinical medicine as well as the research should include intestinal parasites in the list. Furthermore, efforts to reduce childhood illnesses should focus on educating women on hygiene promotion for all age groups especially children under- five year.

The following recommendations are made to:

i. The provincial hospital should promote the community health education on diarrheic diseases especially parasitic infestations and also should implement laboratory techniques other than the direct microscopic examination

ii. The Government should increase the efforts on prevention of childhood illnesses including initiating anti-parasitic programs for under-five children in all health facilities including educating women on hygiene promotion.

iii. Researchers should continue to investigate on the causes of intestinal parasitic infestations and to give public health orientation on their prevention measures.

REFERENCES

Ahmed, S. F., Farheen, A., Muzaffar, A. and Mattoo, G. M. (2008). Prevalence of diarrheal disease, its seasonal and age variation in under - fives in Kashmir, India. *International Journal Health Science* 2: 126 - 133.

Beyene, G. and Haile-Amlak, A. (2004). Antimicrobial sensitivity Pattern of Campylobacter species among children in Jimma University Specialized Hospital, Southwest Ethiopia. *Ethiopian Journal Health Development* 19(1): 185 - 186.

Birmingham, M. E., Lee, L. A., Ntakibirora, M., Bizimana, F. and Deming, M. S. (1997). A household survey of dysentery in Burundi: implications for the current pandemic in sub-Sahara Africa. *Bulletin World Health Organization* 75(1): 45 - 53.

Centres for disease Control and Prevention Diarrhea (2020). Common illness, global killer. [https://www.cdc.gov/healthywater/pdf/global/programs/Globaldiarr hea 508c. pdf]. Site visited on 17/04/2020.

Diouf, K., Tabatabai, P., Rudolph, J. and Marx, M. (2014). Diarrhea prevalence in children under five years of age in rural Burundi: An assessment of social and behavioral factors at the household level. *Global Health Action* 7(1): 24-35.

Eshetu, G. and Kefiyalew, J. (2018). Prevalence and factors associated with intestinal parasitic infestation among under-five children in and around Haro Dumal

Town, Bale zone, Ethiopia. Federal Democratic Republic of Ethiopia. National Hygiene and Sanitation Strategy Ministry of Health. 85pp.

Frederick, O. A., Christopher, E. O. and Richard, O. (2011). Seasonal Variation of intestinal parasitic infestations among HIV positive patients in Benin City, Nigeria. *Ethiopian Journal of Health Sciences* 24(5): 9 - 20.

Huang, D. B. and White, A. C. (2006). An updated review on *Cryptosporidium* and *Giardia*. *Gastroenterology Clinics* 35(2): 291 - 314.

ISTEEBU, MSPLS and ICF International (2012). Demographic and Health Survey, 2010, Institut des Statistiques et d'Etudes Economiques du Burundi, Ministere de la Sante Publique et de Lutte contre le Sida, ICF International, Bujumbura, Burundi. 419pp.

IVAC (2014). *Diarrhea Progress Report. International Vaccine Access Center*. Johns Hopkins Bloomberg School of Public Health. 5pp.

Jaran, A. S. (2016). Prevalence and seasonality variation of human intestinal parasites in patients attending hospital with abdominal symptoms in Northern Jordan. *Eastern Mediterranean Health Journal* 22(10): 756 - 760.

Kaur, R., Rawat, D., Kakkar, M., Uppal, B. and Sharma, V. K. (2002). Intestinal parasites in children with Diarrhea in Delhi, India. *Southeast Asian Journal of Tropical Medicine and Public Health* 33(72): 5 - 9.

Keddy, K. H., Smith, A. M. and Page, N. A. (2016). GEMS extend understanding of childhood diarrhea. *Lancet* 388(10051): 1252 - 1254.

Kotloff, K. L. (2017). The burden and aetiology of diarrheal illness in developing countries. *Pediatric Clinic* 64(4): 799 - 814.

Mahfouz, A. A. R., El-Morshedy, H., Farghaly, A. and Khalil, A. (1997). Ecological determinants of intestinal parasitic infestations among pre-school children in an Urban Squatter Settlement of Egypt. *Journal of Tropical Pediatric* 43(3): 41 - 44.

Mandomando, I. M., Macete, E. V., Ruiz, J., Sanz, S., Abacassamo, F. and Vallès, X. (2007). Etiology of diarrhea in children younger than 5 years of age attending in a rural hospital of Southern Mozambique. *American Journal of Tropical Medicine and Hygiene* 76(7): 522 - 525.

Mekonnen, H. S. and Ekubagewargies, D. T. (2019). Prevalence and factors associated with intestinal parasites among under-five children attending Woreta Health Center, Northwest Ethiopia. *Bio Med Central Infectious Diseases* 19(1): 256 - 258.

Mulatu, G., Zeynudin, A., Zemene, E., Debalke, S. and Beyene, G. (2015). Intestinal parasitic infestations among children under five years of age presenting with diarrheal diseases to two public health facilities in Hawassa, South Ethiopia. *Infectious Diseases of Poverty* 4(1): 49 - 51.

Mulatya, D. M. and Ochieng, C. (2020). Disease burden and risk factors of diarrhea in children under five years: Evidence from Kenya's demographic health survey. *International Society for Infectious Diseases* 93: 359 - 366.

Nathan, C., Jedidiah, S., David, G., Sam, H. and Jason, R. (2018). Deworming in pre-school age children global empirical analysis of health outcomes. *PLoS Neglected Tropical Diseases* 12(4): 5 - 10.

Okyay, P., Ertug, S., Gultekin, B., Onen, O. and Beser, E. (2004). Intestinal parasites prevalence and related factors in school children, a western city sample-Turkey. *Bio Medical Central Public Health* 4(1): 64 - 69.

Platts-Mills, J. A., Babji, S., Bodhidatta, L., Gratz, J., Haque, R. and Havt, A. (2015). Pathogen-specific burdens of community diarrhea in developing countries: A multisite birth cohort study (MAL-ED). *Lancet Global Health* 3(9): 564 - 575.

Quigley, M. A., Kelly, Y. J. and Sacker, A. (2007). Breastfeeding and hospitalization for diarrheal and respiratory Infestation in the United Kingdom millennium cohort study. *Pediatrics* 119(4): 837 - 842.

Quihui, L., Valencia, M. E., Crompton, D. W. T., Phillips, S., Hagan, P. and Morales, G. (2006). Role of the employment status and education of mothers in the prevalence of intestinal parasitic infestations in Mexican rural school children. *Biomedical Central Public Health* 6(1): 225 - 232.

Smith, H. V., Caccio, S. M., Cook, N., Nichols, R. A. B. and Tait, A. (2007). *Cryptosporidium* and *Giardia* as foodborne zoonoses. *Veterinary Parasitology* 149(2): 29 - 40.

Strausbaugh, L. J. and Herwaldt, B. L. (2000). *Cyclospora cayetanensis*: a review, focusing on the outbreaks of *cyclosporiasis* in the 1990s. *Clinical Infectious Diseases* 31(4): 1040 - 1057.

Tellevik, M. G., Moyo, S. J., Blomberg, B., Hjøllo, T., Maselle, S. Y., Langeland, N. and Hanevik, K. (2015). Prevalence of *Cryptosporidium parvum/hominis, Entamoeba histolytica* and *Giardia lamblia* among young children with and without Diarrhea in Dar es Salaam, Tanzania. *PLoS Neglected Tropical Diseases* 23(10): 25 - 41.

Uganda Bureau of Statistics Kampala and Uganda Demographic and Health Survey (2016). Key indicators 169pp. [https://dhsprogram.com/pubs/pdf/FR333/FR333.pdf]. Site visited on 20/5/2018.

UNICEF (2018). [http://www.data.unicef.Org/topic/child-health/diarrhea-disease]. Site visited on 20/5/2018.

UNICEF/WHO (2009). Why Children Are Still Dying and What Can Be Done. United Nations Children's Fund, Geneva, Switzerland 12 - 29.

UNIGME (2018). Levels and Trends in Child Mortality. United Nations Children’s Fund, New York, USA. 48pp.

Vargas, M., Gascon, J., Casals, C., Schellenberg, D., Urassa, H., Kahigwa, E., Ruiz, J. and Vila, J. (2004). Etiology of diarrhea in children less than five years of age in Ifakara, Tanzania. *American Journal Tropical Medicine Hygiene* 70(5): 536 - 539.

Wagner, E. G. and Lanoix, J. N. (1958). Excreta disposal for rural areas and small communities (1958). *Monograph Series World Health Organization* 39: 1 - 182.

WGO (2012). Acute Diarrhea in Adults and Children. *A Global Perspective Milwaukee*. World Gastroenterology Organization, 24pp.

WHO (2002). Reducing risks, promoting healthy life. World Health Organization, England 16pp.

WHO (2013). Diarrhea disease key facts (2013). [http://www.who.int/en/news-room/fact-sheets/detail/diarrhea disease]. Site visited on 23/10/2012.

Zhang, S. X., Zhou, Y. M., Xu, W., Tian, L. G., Chen, J. X. and Chen, S. H. (2016). Impact of co-infestations with enteric pathogens on children suffering from acute diarrhea in South west China. *Infectious diseases of Poverty* 5(1): 1- 13.

APPENDICES

Appendix 1: Questionnaire

Section A: Socio - demographic information

Identification of Participants

1. Name of child.

2. Origin.

3. Gender of the child

    a) Female

    b) Male

4. How old is your child?

5. Number of children in household

6. Marital status of the parents

    a) Married

    b) Single mother

    c) Divorced.

    d) Widower

Section B. Family socio-economic status

7. Which is your source of income?

    a) Agriculture

    b) Business

    c) Employed

    d) Unemployed

8. What is the educational level of the care giver of the child?

    a) Informal

    b) Primary

c) Secondary

d) Other (specify)

Section C: Knowledge of Participant about Diarrhea Infestation

9. What is diarrhea infestation?

10. Have you heard diarrhea?

a) Yes

b) No

11. Have you experienced intestinal parasite to the children?

a) Yes

b) No

12. If yes, where did you hear?

a) On my self

b) On my Children

c) To my friends/ Family

d) In the hospital

e) In the radio/Television / News paper

13. What were the symptoms?

a) Undulant fever

b) Abdominal pain

c) Loss of appetite

d) Vomiting.

e) Weight loss

f) Others (Specify).

14. Have you visited the hospital?

    a) Yes

    b) No

15. What are the main risk factors for diarrhea infestation?

16. What are the transmission mode of intestinal parasites?

17. Among these age group, what are the most vulnerable of diarrhea infestation?

    a) 0-6 months

    b) 7-11months

    c) 12-23months

    d) 24-35months

    e) 36-47months

    f) 48-59 months

18. What are the main measures of prevention of intestinal parasites?

19. When intestinal parasites are very common (During rain Season or Dry season?)

20. Did your children give them antiparasitic?

    a) Yes

    b) No

21. If yes, after how long time did you give that?

Section D: Risk factors associated with intestinal parasites infestation

22. Does your child eat raw fruit or vegetables?

    a) Yes, always

    b) Yes, sometimes

    c) No

23) Do you wash them before eating?

a) Yes

b) Non

24. Does the infected children being separated from others (Home or Hospital)?

a) Yes

b) No

25. What are the source of drinking water?

a) River water

b) Water from rain

c) Water from lake

d) Tap water

e) Others (Specify)

26. Do you have a habit of treating the water?

a) Yes

b) No

27. If yes, what do you usually do to make the water safe to drink?

a) Boiling

b) Filtering

c) Others (specify)

28. Do you have latrine?

a) Yes

b) No

29. Ownership of the latrine

a) Private

b) Shared with neighbours

30. How is hygienic situation of your kids? (Body and clothes)

a) Better

b) Intermediate

c) Poor

31. How is the situation of children playground cleanliness?

a) Better

b) Intermediate

c) Poor

A. TAARIFA YA KIJAMII NA IDADI YA WATU NA UTAMBULISHO WA WASHIRIKI

1. Jina la mtoto

2. Asili

3. Jinsia ya mtoto

a. Mwanamke

b. Mwanaume

4. Mtoto wako ana umri gani?

5. Idadi ya watoto katika kaya

6. Hali ya ndoa ya wazazi

a. Wanandoa

b. Mama asiye na mume

c. Waliachana

d. Mjane hali ya kijamii na kiuchumi ya familia

7. Chanzo chako cha mapato ni kipi?

a. Kilimo

b.Biashara

c. Kuajiriwa

d. Kutokuwa na ajira

8. Kiwango cha elimu cha malezi ya mtoto ni kipi?

a. Sekondari

b. msingi

c. chuo

d. Nyingine (taja)

B**. SEHEMU YA MAARIFA YA MSHIRIKI KUHUSU UGONJWA WA KUHARA**

9. Ugonjwa wa kuhara ni nini?

10. Je, kuhara ni hatari?

b. Ndio

c. Hapana

11. Uzoefu matumbo vimelea kwa watoto?

a. Ndio

b. Hapana

12. Kama ndio, ambapo ulisikia?

a. Kwa mie

b. Kwangu

c. Mtoto wangu

d. Kwa marafiki zangu

e. Familia

f. Katika hospitali

g. Katika redio/ televisheni

h. News

13. Ulikuwa na dalili?

a. Homa undulant

b. Maumivu ya tumbo

c. Kupoteza hamu ya chakula

d. Vomiting

e. Kupungua uzito

f. Wengine

14. Je alitembelea hospital

a.Ndio

b. Hapana

15. Je, ni dalili zipi kuu za hatari za kuhara infestation?

16. Je, ni maambukizi gani ya vimelea intestinal?

17. Miongoni mwa hawa rika, ni mazingira magumu zaidi ya infestation kuhara ?

a. 0-6m

b. 7-11m

c.12-23m

d.24-35m

e. 36-47m

f. 48-59m

18. Ni hatua kuu zipi za kuzuia vimelea intestinal?

19. Nini wakati gani vimelea huwa ni vya kawaida sana

20. Je watoto wako huwapa antiparasitic?

    a. Ndio

    b. Hapana

21. Kama ndio, baada ya muda gani wakati gani kutoa kwamba sehemu?

C. SABABU ZA HATARI ZINAZOHUSIANA NA UVAMIZI WA VIMELEA VYA MATUMBO?

22. Je, mtoto wako hula matunda ghafi au mboga?

    a. Ndiyo, mara zote

    b. Ndiyo, mara chache

    c. Hapana

23. Je, safisha yao kabla ya kula?

    a. Ndio

    b. Hapana

24. Je watoto walioambukizwa hutengwa na wengine (nyumbani au hospital)

    a. Ndio

    b. Hapana

25. Je, nini chanzo cha maji ya kunywa?

    a. Maji ya mto (river water)

    b. Maji kutoka mvua (rain)

    c. Water kutoka ziwa (lake)

    d. Maji ya bomba (tape)

    e. Mengine (others)

26. Je, una tabia ya kutibu maji?

a. Ndio

b. Hapana

27. Kama ndio, huwa unafanya nini ili kufanya maji kuwa salama kwa kunywa?

a. Kuchemsha (Boiling)

b. Kuchuja (Filtering)

c. Mengine (others)

28. Je, una choo?

a. Ndio

b. Hapana

29. Umiliki wa choo

a. Private

b. Kushirikiana na majirani

30. Je, hali ya usafi ya watoto wako ikoje?

a. Bora

b. Kati

c. Maskini

31. Je, hali ya watoto usafi wa uwanja wa michezo ikoje ?

a. Bora zaidi

b. Kati

c. Duni